RHIZOCTONIA SOLANI DAMPING-OFF OF SUGARBEET: EFFECT OF PLANT GROWTH STAGE ON DISEASE SEVERITY AND MANAGEMENT USING PENTHIOPYRAD

A Thesis
Submitted to the Graduate Faculty
Of the
North Dakota State University
Of Agriculture and Applied Sciences

By

Yangxi Liu

In Partial Fulfillment of the Requirements
For the Degree of
MASTER OF SCIENCE

Major Department:
Plant Pathology

April 2015

Fargo, North Dakota

North Dakota State University

Graduate School

Title

Rhizoctonia solani Damping-off of Sugarbeet: Effect of Plant Growth on Disease Severity and Management using Penthiopyrad

By

Yangxi Liu

The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

SUPERVISORY COMMITTEE:

Mohamed Khan
Chair

Marisol Berti

Luis del Rio

Melvin Bolton

Approved:

8-14-2015	Jack Rasmussen
Date	Department Chair

ABSTRACT

Rhizoctonia solani is the most damaging pathogen on sugarbeet (*Beta vulgaris* L.) in North Dakota and Minnesota. Research was conducted to evaluate penthiopyrad for controlling *R. solani* and determine when the plants are most susceptible to infection. Penthiopyrad applied in-furrow and as a soil drench resulted in significantly higher percent survival than the positive control whereas penthiopyrad applied in a band was ineffective at controlling *R. solani.* Penthiopyrad can also be used as a seed treatment at the 14 g rate to provide effective control of *R. solani*. Sugarbeet plants, irrespective of their inherent level of resistance, were easily infected by *R. solani* up to three weeks after planting, even longer for susceptible varieties, highlighting the need for additional protection in the form of seed treatment or fungicide application that may be required to protect vulnerable sugar beet planted in fields with a history of the disease.

ACKNOWLEDGEMENTS

I would like to thank my advisor Dr. Mohamed Khan for his guidance, encouragement, support, and patience throughout my research. Also, I extend my thanks to the graduate committee Drs. Luis del Rio, Melvin Bolton, and Marisol Berti for their help, support and valuable comments and suggestions to improve my research. Additionally, I would like to thank all faculty members, staff, and graduate students of the Plant Pathology Department for their kindness and support. I am thankful for all people helped me throughout my research especially Sahar Arabiat and Peter Hakk. Also I am thankful for Dr. Kishore Chittem for his help in data analysis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1. LITERATURE REVIEW

Sugarbeet history and development

Sucrose, commonly referred to as sugar, is obtained from two crops, sugarcane (*Saccharum officinarum*) and sugarbeet (*Beta vulgaris* L.). Sugarcane has been and is still cultivated in the tropical regions for many centuries, while sugarbeet is a relatively new crop with the first commercial production in temperate regions in the 19th century. Sugarbeet has a relatively short growing season in temperate regions to produce roots with a high concentration of sucrose. These characteristics make sugarbeet an important crop for supplying sucrose in most temperate parts of the world (Draycott, 2006).

The first milestone of the modern sugarbeet industry was made in 1747 by Andreas Sigismund Marggraf, who discovered sugar crystallization from sugarbeet juice. Forty years later, Marggraf's student Franz Carl Achard, bred the White Silesian beet which is the ancestor of sugarbeet with characterized white skin roots, white flesh, and a conical shape. He also developed sugar extraction process for harvested beets and established the world's first sugarbeet factory in 1801. An initial stimulus was given to sugarbeet industry in 1811 when Napoleon I expanded on Achard's research and incentivized French growers to build factories for processing sugarbeet as an alternative to sugarcane in order to minimize the effect of the British Blockade. In the 19th century, various technological developments as well as favorable government policies helped the sugarbeet industry to expand throughout Europe to countries in North and South America, Asia, and Africa (Francis, 2006).

In the United States, the first sugarbeet factory was established in Massachusetts in 1938, but the first successfully-operated one was founded at Alvarado in California in 1870 (Francis, 2006). Currently, sugarbeet plants are produced in ten states including California, Colorado, Idaho,

Michigan, Minnesota, Montana, Nebraska, North Dakota, Oregon, and Wyoming (USDA-ERS 2014). In the Red River Valley of Minnesota and North Dakota, the first beet-processing factory was built in East Grand Forks in 1926, marking the beginning of large-scale sugarbeet production in these states. Today, there are seven processing factories in Minnesota and North Dakota managed by three producer-owned cooperatives: American Crystal Sugar Company, Minn-Dak Farmers Cooperative, and Southern Minnesota Beet Sugar Cooperative. In 2010, Minnesota and North Dakota accounted for 57% of the nation's sugarbeet acreage, producing 55% of the nation's sugarbeet tonnage. In 2011, sugarbeet production, processing, and market activities contributed $ 1.7 billion to the economy of the two states (Bangsund et al., 2012).

In 2012, the Russian Federation, France, the United States, Germany, and Turkey were the world's five largest sugarbeet producers with 153.6 million metric tons of sugarbeet. The United States was the second largest producer with around 31.9 million metric tons from 1.2 million acres at that time (FAO, 2012).

However, diseases such as Rhizoctonia crown and root rot (*Rhizoctonia solani* Kühn), Fusarium yellows (*Fusarium oxysporum* f. sp. *betae* Snyder & Hansen), Aphanomyces root rot (*Aphanomyces cochlioides* Drechsler), Cercospora leaf spot (*Cercospora beticola* Saccardo), and Rhizomania (beet necrotic yellow vein virus transmitted by *Polymyxa betae* Keskin) are endemic and limit profitable sugarbeet production in the Red River Valley of Minnesota and North Dakota. *Rhizoctonia solani* is a common, soil-borne fungal pathogen causing damping-off, Rhizoctonia crown and root rot on sugarbeet worldwide. This disease has become more prevalent and severe in the Red River Valley and was listed as the most serious problem for sugarbeet production by growers in Minnesota and North Dakota since 2009 (Stachler et al., 2009).

Rhizoctonia crown and root rot

Rhizoctonia damping-off, and crown and root rot are caused by the soil-borne fungus *Rhizoctonia solani* found wherever the crop is grown worldwide (Ogoshi, 1987). Losses due to *R. solani* average 2% annually, but 24% of the sugarbeet growing acreage in the United States was affected (Windels et al., 2009). Yield loss caused by *R. solani* varies greatly and there can be more than 50% yield loss when not controlled in heavily infested fields (Büttner et al., 2004; Khan et al., 2010; Windels and Brantner, 2005).

Damping-off causes death of seedlings resulting in a reduced plant population early in the season which may reduce yields. Crown and root rot typically damage the root leading to the reduction of sucrose extraction from the roots where most of the sucrose is stored (Cooke and Scott, 1993). Due to infection by *R. solani*, cracks and fissures develop on the root surface which may serve as entry wounds for other organisms that can cause more damage to roots. After harvest, these injured roots in the storage piles can lead to hot spots that may result in storage loss and processing difficulties (Gallian, 2001).

Description of *Rhizoctonia solani*

Rhizoctonia solani Kühn (teleomorph, *Thanatephorus cucumeris* (A. B. Frank) Donk), first described by Julius Kühn on potato in 1858, is the ubiquitous soil-borne fungi in the Domain Eukaryota; Kingdom: Fungi; Phylum: Basidiomycota; Order: Ceratobasidiales; Family: Ceratobasidiaceae; Genus: *Rhizoctonia* (Agrios, 2005).

The genus *Rhizoctonia* does not produce asexual spores, but initially exists as sclerotia within the hosts and germinates a hyaline sterile mycelium. *Rhizoctonia solani* is a saprotrophic and facultative plant pathogen, and its cell contains multiple nuclei (Multinucleate *Rhizoctonia*) (Agrios, 2005). It produces colorless mycelium but becomes yellowish or light brown over time.

The mycelium branches at right angle to the main hyphae, and a slight constriction is present at the hyphal branch, which are the main identifying characteristics of *R. solani* (Harveson et al., 2009; Whitney and Duffus, 1986). The teleomorphic state of *R. solani* (*Thantephorus cucumeris*) occasionally develops in conditions of high relative humidity to form barrel-shaped to sub-cylindrical basidia with four apical sterigmata, on which a smooth, thin-walled, apiculate, ovate, and hyaline basidiospore (Asher and Hanson, 2006; Herr, 1981). In 1993, basidiospores of *T. cucumeris* was found on sugarbeet near East Grand Forks, Minnesota (Windels et al., 1997).

Rhizoctonia solani is composed of different genetically isolated populations called anastomosis groups (AGs). This classification is based on compatible hyphal fusion that occurs when two *R. solani* isolates belongs to the same AG (Agrios, 2005). When hyphal fusion does not occurs or lead to 'killing reaction' between two isolates, they are considered to be genetically different. However, the genetic mechanism behind this recognition process among *R. solani* anastomosis groups is not well understood. Fourteen AGs have been identified and described in *R. solani*. (Carling, 1996; Gonzales Gracia et al., 2006; Stodart et al., 2007). Subdivision of AG is based on their different performance in virulence, hyphal fusion frequency, and effect of temperature on growth (Carling et al., 2002; Vigalys and Cubeta, 1994).

Distribution and host range

In Minnesota and North Dakota, *R. solani* AG 1, AG 2-2, AG 4, and AG 5 were isolated from sugarbeet samples: AG 1, AG 2-2, and AG 4 cause damping-off of seedlings; AG 2-2 was also pathogenic to mature plants resulting in crown and root rot (Windels and Nabben, 1989); AG 3 and AG 5 were non-pathogenic or resulted in few symptoms (Windels et al., 1997).

R. solani AG 2-2 IIIB and AG 2-2 IV, the two subgroups of *R. solani* AG 2-2, are the most destructive to sugarbeet. These two subgroups can be distinguished by the ability to grow at 35°C:

AG 2-2 IIIB grows and survives at 35°C, whereas AG 2-2 IV does not (Sneh et al., 1991). The AG 2-2 IIIB is considered to be more aggressive than AG 2-2 IV (Bolton et al., 2010; Engelkes and Windels, 1994). In the northern Red River Valley, 66% of the sugarbeet with Rhizoctonia crown and root rot was caused by *R. solani* AG 2-2 IV, followed by 27% for AG 2-2 IIIB, and 7% for unidentified subgroups; in Southern Minnesota, 23% of diseased samples were attributed to AG 2-2 IV, 56% to AG 2-2 IIIB with 21% for unidentified subgroups (Brantner and Windels, 2007).

Disease symptoms

Rhizoctonia solani induces different types of disease symptoms on sugarbeet depending on its growth stage. Seedling disease symptoms like seed rot and damping-off are difficult to be distinguished from the symptoms caused by other soil-borne pathogens, especially *Aphanomyces cochlioides* Drechsler, *Pythium aphanidermatum* (Edson) Fitzpatrick, and *Phytophthora drechsleri* Tucker (Herr, 1996). Damping-off usually occurs after emergence, and infection begins with brown to black discolorations on the hypocotyl. Seedlings die when the hypocotyl is severely damaged (Whitney and Duffus, 1986). Crown rot is typically due to the deposition of *R. solani*-infested soil onto the crown during cultivation practices or from heavy rains or flooding (Schneider et al., 1982). Root rot causes black to brown, sunken, circular lesions that cover the root surface until the advanced stages of the disease, when it moves interiorly. Cracks and splits are common on the crown area and on the side of infected roots, where brownish fungal hyphae may be visible (Harveson et al., 2009; Neher and Gallian, 2011). The above-ground symptoms of Rhizoctonia crown and root rot include wilting and chlorosis of leaves, stunting, and blackened lesions at the base of petioles. Finally, wilted leaves collapse onto the soil surface forming a dry, brown or black rosette, but are still attached to the crown (Asher and Hanson, 2006; Whitney and Duffus, 1986).

Disease cycle and infection process

Rhizoctonia solani overwinters in the soil and plant debris as hyphae fragments, sclerotia, or bulbils. Bulbils are dry and hard survival structures, resembling sclerotium measuring 0.1 to 10 mm in diameter. The dormant state of *R. solani* becomes active in warm temperature. *R. solani* initiates infection on sugarbeet under a wide temperature range from 13 to 35°C. The optimal range is between 20°C and 30°C, but infection occasionally occurs below 15°C (Bolton, 2010; Leach, 1986). The disease can develop in the soils with 25% moisture-holding capacity, and high soil moisture will increase disease severity (Bolton et al., 2010). Root exudates from sugarbeet can activate sclerotia germination or mycelial growth of *R. solani*. This fungus is more attracted to exudates from young hosts than from old hosts (Gonzales Gracia et al., 2006). When the hyphae grows over and attaches to the root surface, the 'T-shaped' infectious cushion is developed, from which an infection peg directly penetrates the host tissue by hydrostatic pressure (Gonzales Gracia et al., 2006; Herr, 1996). The fungus can penetrate the host through direct penetration, natural openings, and wounds. During penetration, *R. solani* secretes several enzymes, such as pectinase, pectin lyase, and cellulase, to breakdown or degrade the plant tissues (Sneh et al., 1996). Also, the pectin lyase alone causes wilting in sugarbeet plants, indicating that it may be associated with *R. solani* pathogenicity (Bugbee, 1990).

Rhizoctonia solani AG 2-2 may occur in all types of soil, but is more severe in field depressions where the soils are heavy and poorly-drained (Whitney and Duffus, 1986). Research shows that the highest inoculum density of *R. solani* appears in the upper 10-cm soil with no fungal activity below 15-cm soil depth (Paula et al., 2008). The pathogen can be disseminated by wind, irrigated water, and infested-soil movement.

Management

Genetic resistance, biological control, cultural practices, and chemical control can be combined to manage *R. solani* depending on the inoculation population in sugarbeet fields.

Genetic resistance

In the late 1950s, Gaskill (1968) started breeding and developing *R. solani*-resistant sugarbeet germplasms in Rhizoctonia artificially-inoculated fields in Fort Collins, Colorado. In 1966, the resistant germplasm lines, FC701 and FC702, were first released, but not acceptable for commercial use. Improved resistant germplasm lines, including FC704, FC706, FC710, FC711, and FC712, were developed afterwards (Campbell and Bugbee, 1993). Some resistant germplasm lines, such as FC716 and FC717, were incorporated into commercial cultivars to achieve a better agronomic performance (Panella et al. 1995). More recently, breeders have combined *R. solani* resistance with the other important disease resistances such as *Aphanomyces cochlioides* Drechsler and *Cercospora beticola* Saccardo in FC1018, FC1019, and FC1020 germplasm lines (Panella, et al., 2011).

No commercial sugarbeet cultivars are immune to infection by *R. solani*. The partially-resistant cultivars have a potential yield loss of 10 to 15% less than susceptible commercial cultivars in the absence of disease (Panella and Ruppel, 1996). These cultivars can be used with timely fungicide applications in the sugarbeet field with a history of *R. solani* occurrence. However, this resistance fails to prevent the stand loss caused by Rhizoctonia damping-off early in the season (Gaskill, 1968). Sugarbeet germplasm SR98 is reported to provide improved seedling resistance to *R. solani* (Mcgrath et al., 2015). Also, the germplasm line EL51 is considered as a

possible source of the *R. solani* damping-off resistance, since the seedlings can survive the inoculation with *R. solani* AG 2-2 (Nagendran et al., 2009).

Biological control

Biological control is an important component of integrated pest management (IPM) program by artificially inducing natural enemies to suppress pest populations.

Plant surface-colonizing biocontrol agents can be used to manage *R. solani* due to their competitive relationship. Rhizobacteria are a root-colonizing bacteria and establish symbiotic relationships with host plants. Antagonist bacteria *Bacillus* strain MSU-127 was used in the mixture with low rate azoxystrobin, resulting in lower disease severity and greater sucrose yield compared with the standardized-dose azoxystrobin alone (Kiewnick et al., 2001). Binucleate *Rhizoctonia* reduced the disease severity of *R. solani* AG 2-2 and AG 4 on seven soybean (*Glycine max* (L.) Merr.) cultivars, but the effect was only significant on the strain AG-4. Since binucleate *Rhizoctonia* were consistently isolated from hypocotyls and roots of soybean, the reduced disease severity might be due to the competition of colonizing the root surface between binucleate *Rhizoctonia* and pathogenic *R. solani* (Khan et al., 2005).

Some biocontrol agents have inhibitory effects on the fungal growth. Beneficial bacteria *Pseudomonas* CMR12a was reported to produce phenazines and cylic lipopeptides that dramatically reduced disease severity of *R. solani* AG 2-2 and AG 4 on dry bean (*Phaseolus vulgaris* L.) plants (D'aes et al., 2001). Barakat et al. (2007) reported that *Thichoderma* spp. applied as a conidial suspension was able to reduce the disease index by 65% on dry bean plants and that *T. harzianum* Rifai and *T. hamatum* (Bonord.) Bainier were the most effective isolates that inhibited *R. solani* mycelial growth at 25°C. Potato (*Solanum tuberosum* L.) tubers treated with *Microsphaeropsis* sp. strain P130A reduced the number of *R. solani* AG-3 sclerotia compared

with an increased number of sclerotia in untreated tubers after 8 months of incubation. In this trial, *Microsphaeropsis* sp. might play a role in cytoplasm disorganization and plasma membranes breakdown of *R. solani* cells (Carisse et al., 2001).

Some fungi might have their potentials to serve as bio-control agents against *R. solani*. Three yeasts, *Candida valida* (Leberle) Uden & H.R. Buckley, *Trichosporon asahii* Akagi ex Sugita, A. Nishikawa & Shinoda, and *Rhodotorula glutinis* (Fresen.) F.C. Harrison, isolated from the rhizosphere of sugarbeet roots, had a synergistic effect on increasing the efficacy of managing *R. solani* AG 2-2. It indicates that there might be different mechanisms available against this pathogen (El-Tarabily, 2004). In *in vitro* assays, *Rhizoctonia zeae* Voorhees was shown to inhibit the radial growth of multiple sugarbeet root pathogens including *R. solani*. This fungus provided some degrees of protection against soil-borne diseases when it was applied in a naturally-infested field, and therefore could be considered as potential bio-control agents (Webb et al., 2015).

Cultural practices

Crop rotation with non-host crops is an effective practice for managing *R. solani* and increasing sugarbeet yield. Different rotation crops resulted in different infestation levels of *R. solani* (Herr, 1987). Planting host crops of *R. solani* AG 2-2 in the rotation will increase the inoculum potentials in the field (Windels and Brantner, 2004). Potato, alfalfa (*Medicago sativa* L.), sweetclover (*Melilotus officinalis* (L.) Pall.), sorghum (*Sorghum bicolor* (L.) Moench), and bean species are considered as host crops of *R. solani* (Maxon, 1938; Rush and Winter, 1990), whereas cereal crops such as wheat (*Triticum* L.), barley (*Hordeum vulgare* L.), and corn (*Zea mays* L.) as non-hosts for *R. solani* AG 2-2. Rotation with cereal crops decreased inoculum population and therefore, recommended for rotation with sugarbeet in the upper Midwest. *Rhizoctonia solani* AG 2-2 IIIB was reported to cause lesions in corn in the southeastern United

States (Sumner, 1982, 1999). Research conducted in Europe revealed that the isolate of *R. solani* AG 2-2 IIIB recovered from root and stalk rot of corn also causes Rhizoctonia crown and root rot on sugarbeet (Ithurrart et al., 2004). Furthermore, *R. solani* AG 2-2 III and IV were isolated from wheat, soybean, and corn roots (Windels and Brantner, 2006). The interval between the cultivation of *R. solani*-susceptible sugarbeet cultivars in the crop rotation is a major factor affecting disease occurrence and severity (Ruppel, 1985). A 3-year minimum rotation with non-host crops is recommended to manage *R. solani* population in the field (Windels and Brantner, 2006; Windels, 1988). However, economic factors, such as marketability and commodity price of different crops, are often more important for growers in terms of selecting rotation crops with sugarbeet. During the last two decades, there was a significant increase in the acreage of soybean and corn susceptible to *R. solani* AG 2-2. This shift in crop production from wheat to soybean and corn coupled with a wet cycle could be an important contributor to the fact that *R. solani* has become more prevalent and severe in Minnesota and North Dakota (Brantner and Windels, 2007).

Soil tillage may affect plant disease occurrence indirectly by altering the biological and physical properties of soils or microbial growth. The organic particles are good dwelling places for *R. solani* to overwinter and to maintain population (Papavizas, 1968). A higher survival rate of *R. solani* in upper soil layers was found compared with lower layers (Ruppel, 1991). Soil tillage helps bury the infested organic tissues into the deeper soil layer, reducing inoculum population in the field. Paulitz et al. (2002) speculated that soil cultivation disrupts the mycelial network of *Rhizoctonia* spp., so the pathogen's vigor is reduced. It was also reported that cereal fields with reduced tillage had a higher infestation level of *R. solani* compared with plowed fields (Paulitz, 2006; Rovira, 1986).

Cultivation practices for weed control are associated with crown rot through the deposition of *R. solani*-infested soil onto the crown (Schneider et al., 1982). This practice is replaced by planting glyphosate-resistant sugarbeet cultivars with timely application of glyphosate to manage weed problems (Khan, 2010). Other practices to manage *R. solani* include early planting in cool soil environments before the pathogen becomes infective (Khan et al., 2008), sanitation to reduce the inoculum density, and improved drainage in the field.

Chemical control

The use of fungicide has been considered as the most reliable method among all control measures against *R. solani* in sugarbeet-growing areas in Minnesota and North Dakota. In the United States, Thiram (Thiram, Bayer CropScience) and Fludioxonil + Mefenoxam (Apron XL, Syngenta) are used as seed treatment in commercial sugarbeet but they are not effective at controlling seed rot and damping-off caused by *R. solani* (Brantner and Windels, 2007). In 2001, azoxystrobin (Quadris, Syngenta), which belongs to quinone outside inhibitor (QoI) fungicide group, was registered for control of *R. solani* on sugarbeet (Jacobsen et al., 2001) and it was widely used as in-furrow or foliar method in 60.4% of sugar beet growing acres between Minnesota and North Dakota (Carlson et al., 2012). Trifloxystrobin (Gem, Bayer Crop Science), pyraclostrobin (Headline, BASF), and prothioconazole (Proline, Bayer Crop Science) were also registered for foliar application to control *R. solani* on sugarbeet (Friskop et al., 2015). Prothioconazole, trifloxystrobin and pyraclostrobin provided effective control of *R. solani* compared with the untreated control, but their efficacy was not consistent and less effective than azoxystrobin (Bolton et al., 2010; Stump et al., 2004). Fungicides need to be applied for controlling diseases before infection occurs because most fungicides are not curative. Azoxystrobin was reported to be ineffective if it is applied after the infection is well-established (Windels and Brantner, 2002).

Jacobsen et al. (2004) reported that that disease control is optimal when the first application of azoxystrobin was made at soil temperatures between 18 and 21°C at the 10-cm depth. Further research showed that *R. solani* infects sugarbeet when the average daily soil temperature reaches 18°C at the 10-cm soil depth (Khan et al., 2010).

Stump et al. (2004) reported that in-furrow application of azoxystrobin effectively provided control of damping-off that occurred early but had no effect on crown and root rot of *R. solani* later in the late season. A single in-furrow application was not enough to provide season-long disease control, especially in the field with high density of *R. solani* population. An in-furrow application combined with band application at the four-leaf stage reduced disease incidence and severity of *R. solani* through the growing season and increased the yield comparable to the untreated control (Windels and Brantner, 2005). Foliar application was usually applied at the four leaf stage since this is the typical time when cultivation occurs, depositing the *R. solani*-infested soils onto the sugarbeet crown to cause infections. Effective disease control by foliar application of azoxystrobin was reported by several researchers (Jacobsen et al., 2004; Kiewnick et al., 2001; Windels and Brantner, 2009).

Growers typically use the QoIs azoxystrobin, and to a lesser extent, pyraclostrobin as in-furrow applications to control Rhizoctonia damping-off followed by a band application of azoxystrobin, and less frequently prothioconazole to control root rot. Some growers use only a band application of azoxystrobin or prothioconazole to control root rot. The continuous use of QoIs, especially azoxystrobin, has resulted in isolates expressing high EC50 values to this product. It will be useful to have other effective modes of action to be used in rotation with QoIs.

Penthiopyrad is a novel succinate dehydrogenase inhibitor (SDHI) class fungicide. The SDHIs targets the complex II electron transport system to interfere the fungal respiration (Avenot

and Michailides, 2010). The first SDHIs, such as carboxin and oxycarboxin, were introduced into the international market since the 1960s but had a limited spectrum of pathogen that they could control. The newer SDHI generation, including boscalid, fluxapyroxad, fluopyram, and penthiopyrad, have a broad spectrum of activity against numerous fungal plant pathogens of various crops (Stammler et al., 2008). Penthiopyrad has displayed its high efficacy against various plant diseases caused by ascomycetes, deuteromycetes and basidiomycetes (Yanase et al., 2007). In the United States, DuPont had the rights to use penthiopyrad as in-furrow or band or foliar applications, whereas Mitsui had the rights to use this product as a seed treatment only. It will be useful to have a new product such as penthiopyrad that can control Rhizoctonia and can be used in rotation with QoI fungicides to prolong the usefulness of both classes of fungicides. It will also be helpful to know exactly when resistance is expressed in sugarbeet so that the most susceptible stages to *R. solani* can be protected with timely application of fungicides.

The objective of this research was 1) to evaluate the efficacy of penthiopyrad as an in-furrow, band application, and soil drench for controlling *R. solani*; 2) to evaluate the efficacy of penthiopyrad as a seed treatment for controlling *R. solani*; 3) and to determine at what age do sugarbeet start to express resistance to *R. solani*.

Literature cited

Agrios, G. N. 2005. Plant Pathology (5th edition). Burlington, MA, USA: Elsevier Academic Press.

Anderson, N.A. 1982. The genetics and pathology of *Rhizoctonia solani*. Annu. Rev. Phytopathol. 20:329-347.

Asher, M.J.C. and Hanson, L.E. 2006. Fungal and bacterial diseases. In: A.P. Draycott (Ed.), *Sugarbeet* (pp.298-299). Blackwell, Oxford, UK.

Avenot, H.F., and Michailides, T.J. 2010. Progress in understanding molecular mechanisms and evolution of resistance to succinate dehydrogenase inhibiting fungicides in phytopathogenic fungi. Crop Prot. 29:643–651.

Bangsund, D.A., Hodur, N.M., and Leistritz, F.L. 2012. Economic contribution of the sugarbeet industry in Minnesota and North Dakota. AAE 668. Dept. of Agribusiness and Applied Economics, North Dakota State Univ., Fargo. Available at http://ageconsearch.umn.edu /bitstream/121494/2/AAE688.pdf. (Verified 11 Mar. 2015).

Barakat, R.M., Al-Mahareeq, F., Ali-Shtayeh, M.S., and Al-Masir, M.I. 2007. Biological control of *R. solani* by indigenous *Trichoderma* spp. isolates from Palestine. Hebron Univ. Res. J. 3:1-15.

Bolton, M.D., Panella, L., Campbell, L., and Khan, M.F.R. 2010. Temperature, moisture, and fungicide effects in managing Rhizoctonia root and crown rot of sugarbeet. Phytopathology 100:689–697.

Brantner, J.R., and Windels, C.E. 2007. Distribution of *Rhizoctonia solani* AG 2-2 intraspecific groups in the Red River Valley and southern Minnesota. SBREB. 38:242–246. Available at http://www.sbreb.org/research/plant/plant07/08DistributionOfRhizocRRV.pdf. (Verified 11 Mar. 2015).

Bugbee, W.M. 1990. Purification and characteristics of pectin lyase from *Rhizoctonia solani*. Physiol. Mol. Plant Path. 36:15-25.

Büttner, G., Pfähler, B., and Märländer, B. 2004. Greenhouse and field techniques for testing sugarbeet for resistance to Rhizoctonia root and crown rot. Plant Breeding. 123:158-166.

Campbell, L.G. and Bugbee, W.M. 1993. Pre-breeding for root-rot resistance. JSBR. 30:241-251.

Carisse, O., El Bassam, S., and Benhamou, N. 2001. Effect of *Microsphaeropsis* sp. strain P130A on germination and production of sclerotia of *Rhizoctonia solani* and interaction between the antagonist and the pathogen. Phytopathology 91:782-791.

Carling, D.E. 1996. Grouping in *Rhizoctonia solani* by hyphal anastomosis reaction. In B. Sneh, S. Jabaji-Hare, S. Neate, & G. Dijst (Eds.), *Rhizoctonia Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control* (pp. 37-47). Kluwer Academic Publishers, Dordrecht, Netherlands.

Carling, D.E., Kuninga, S., and Brainard, K.A. 2002. Hyphal anastomosis reactions, rDNA-internal transcribed spacer sequences, and virulence levels among subsets of *Rhizoctonia solani* anastomosis groups-2 (AG-2) and AG-BI. Phytopathology 92:43-50.

Carlson, A.L., Khan, M.F.R., Stachler, J.M., and Boetel, M.A. 2012. Survey of fungicide use in sugarbeet in Minnesota and eastern North Dakota in 2012. SBREB. 42:181-182. Available at http://www.sbreb.org/research/plant/plant11/CarlsonSurveyFungicide2012.pdf. (Verified 11 Mar. 2015).

Cooke, D.A., and Scott, R.K. (Eds.). 1993. *The Sugarbeet Crop*. Chapman and Hall, London.

D'aes, J., Hua, G.K.H., De Maeyer, K., pannescoucque, J., Forrez, I., Ongena, M., Dietrich, L.E.P., Thomashow, L.S., Mavrodi, D.V., and Hofte, M. 2001. Biological control of Rhizoctonia root rot on bean by phenazine- and cyclic lipopeptide-producing *Pseudomonas* CMR12a. Phytopathology 101: 996-1004.

Draycott, A.P. 2006. Introduction. In A.P. Draycott (Ed.), *Sugarbeet* (pp. 1-8). Blackwell, Oxford, UK.

El-Tarabily, K.A. 2004. Suppression of *Rhizoctonia solani* diseases of sugarbeet by antagonistic and plant growth-promoting yeasts. J. Appl. Microbiol. 96: 69-75.

Engelkes, C.A., and Windels, C.E. 1994. Relationship of plant age, cultivar, and isolate of *Rhizoctonia solani* AG 2-2 to sugarbeet and crown rot. Plant Dis. 78:685-689.

FAO. 2012. Food and agricultural organization statistics. Available at http://faostat.fao.org/site /339/default.aspx. (Verified 11 Mar. 2015).

Francis, S.A. 2006. Development of sugarbeet. In A.P. Draycott (Ed.), *Sugarbeet* (pp. 9-19). Blackwell, Oxford, UK.

Gallian, J.J. 2001. Management of sugarbeet root rot. Pacific Northwest Extension Publication PNW 538.

Gaskill, J.O. 1968. Breeding for Rhizoctonia resistance in sugarbeet. J. Am. Soc. Sugarbeet Technol. 15: 107-119.

Gonzales Gracia, V., Portal Onco, M. A., and Rubio S.V. 2006. Review, biology and systematic of the form genus *Rhizoctonia*. Span. J. Agr. Res. 4:55-79.

Friskop, A., Markell, S.G., Khan, M.F.R., Gudmestad, N., and Secor, G. 2015. 2015 North Dakota Field Crop Plant Disease Management Guide. PP-622 (Revised). North Dakota State Univ. Ext. Serv., ND, USA.

Harveson, R.M., Hanson, L.E., and Hein, G.L. (Eds.). 2009. *Compendium of Beet Diseases and Pests*. The American Phytopathological Society, St. Paul, MN, USA.

Herr, L.J. 1981. Basidial stage of *Rhizoctonia solani*, anastomosis groups 2 and 4, on sugarbeet in Ohio (abstract). Phytopathology 71:224.

Herr, L. J. 1987. Population of *Rhizoctonia solani* in soil under crops in rotation with sugarbeet. Ann. Appl. Biol. 110:17-24.

Herr, L.J. 1996. Sugarbeet diseases incited by *Rhizoctonia solani* spp. In B. Sneh, S. Jabaji-Hare, S. Neate, and G. Dijst (Eds.), *Rhizoctonia Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control* (pp. 341-349). Kluwer Academic publishers, Dordrecht, Netherlands.

Ithurrart, M.E.F., Büttner, G., and Petersen, J. 2004. Rhizoctonia root rot in sugarbeet (*Beta vulgaris* ssp *altissima*)-Epidemiological aspects in relation to maize (*Zea mays*) as a host plant. J. Plant Disease Protection 111:302-312.

Jacobsen, B., Kephart, K., Zidack, N., Johnston, N., and Ansley, J. 2004. Effect of fungicide and fungicide application timing on reducing yield loss to Rhizoctonia crown and root rot. SBREB. 35:224-226. Available at http://www.sbreb.org/04/Pathology%2004/Pathology8.pdf. (Verified 11 Mar. 2015).

Jacobsen, B., Zidack, N., Kephart, K., Mickelson, J., and Ansley, J. 2001. Integrated management strategies for Rhizoctonia crown and root rot. SBREB. 32:293. Available at http://www.sbreb.org/research/plant/plant01/Integrated_Mgmt_Strategies_for_Rhizoctonia_Crown_and_Root_Rot.doc. (Verified 11 Mar. 2015).

Khan, F.U., Nelson, B.D., and Helms, T.C. 2005. Greenhouse evaluation of binucleate Rhizoctonia for control of *R. solani* in soybean. Plant Dis. 89:373-379.

Khan, M.F.R. 2010. Introduction of glyphosate-tolerant sugarbeet in the United States. Outlooks Pest Manage. 21:38-41.

Khan, M.F.R., Khan, J., and Bradley, C.A. 2010. Effect of azoxystrobin applications based on soil temperature on Rhizoctonia root and crown rot of sugarbeet. Int. Sugar J. 112:557-560.

Khan, M.F.R., Qandah, I., and Bolton, M.D. 2008. The effect of temperature on Rhizoctonia disease development and fungicide efficacy in controlling Rhizoctonia root rot on sugarbeet. SBREB. 39:252-254. Available at http://www.sbreb.org/research/plant/plant08/TemperatureRhizoctonia.pdf. (Verified 11 Mar. 2015).

Kiewnick, S., Jacobsen, B.J., Braun-Kiewnick, A., Eckhoff, J.L.A., and Bergman, J.W. 2001. Integrated control of Rhizoctonia crown and root rot of sugarbeet with fungicides and antagonisitic bacteria. Plant Dis. 85:718-722.

Leach, L.D. 1986. Seedling diseases. In: E.D. Whitney and J.E. Duffus (Eds.), *Compendium of Beet Diseases and Insects* (pp. 4-8). The American Phytopathological Society Press, St. Paul, MN, USA.

Maxson, A.C. 1938. Root-rots of the sugarbeet. Proc. Am. Soc. Sugarbeet Technol. 1:60-64.

Mcgrath, J.M., Hanson, L.E., and Panella, L. 2015. Registration of SR98 Sugarbeet Germplasm with Resistances to Rhizoctonia Seedling and Crown and Root Rot Diseases. J. of Plant Regis. 9:227-231.

Nagendran, S., Hammerschmidt, R., and McGrath, J.M. 2009. Identification of sugarbeet germplasm EL51 as a source of resistance to post-emergence Rhizoctonia damping-off. Eur. J. Plant Pathol. 123:461-471.

Neher, O. T. and Gallian, J. J. 2011. Rhizoctonia on sugarbeet. PNW 629. University of Idaho, ID, USA.

Ogoshi, A. 1987. Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani* Kühn. Annu. Rev. Phytopathol. 25:125-143.

Panella, L., Lewellen, R.T., Webb, K.M. 2011. Registration of FC1018, FC1019, FC1020, and FC1022 multigerm sugarbeet pollinator germplasms with disease resistance. J. Plant Regist. 5:233-240.

Panella, L., and Ruppel, E.G. 1996. Availability of germplasm for resistance against Rhizoctonia Spp. In B. Sneh, S. Jabaji-Hare, S. Neate, & G. Dijst (Eds.), *Rhizoctonia Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control* (pp. 515-527). Kluwer Academic publishers, Dordrecht, Netherlands.

Panella, L., Ruppel, E.G., and Hecker, R.J. 1995. Registration of four multigerm sugarbeet germplasms resistant to Rhizoctonia root rot: FC 716, FC 717, FC 718, and FC 719. Crop Sci. 35:291-292.

Papavizas, G.C. 1968. Survival of rootinfecting fungi in soil. VIII. Distribution of *Rhizoctonia solani* in various physical fractions of naturally and artificially infested soils. Phytopathology 58:746-751.

Paula, T.J., Rotter, C., Hau, B. 2008. Effects of inoculum depth on the activity of *Rhizoctonia solani* and on bean root rot development in sterile soil. J. Plant Dis. Protect. 115:172-177.

Paulitz, T.C. 2006. Low input no-till cereal production in the pacific northwest of the U.S.: the challenges of root diseases. Eur. J. Plant Pathol. 115:271-281.

Paulitz, T.C., Smiley, R.W., and Cook, R.J. 2002. Insights into the prevalence and management of soilborne cereal pathogens under direct seeding in the Pacific Northwest, U.S.A. Can. J. Plant Pathol. 24:416-428.

Rovira, A.D. 1986. Influence of crop rotation on Rhizoctonia bare batch of wheat. Phytopathology 76:669-673.

Ruppel, E.G. 1985. Susceptibility of rotation crops to a root rot isolate of *Rhizoctonia solani* from sugarbeet and survival of the pathogen in crop residues. Plant Dis. 69:871-873.

Ruppel, E.G. 1991. Survival of *Rhizoctonia solani* in fallow field soil and buried sugarbeet roots in three depths. JSBR. 28:141-153.

Rush, C.M., and Winter, S.R. 1990. Influence of previous crops on Rhizoctonia root and crown rot of sugarbeet. Plant Dis. 74:421-425.

Schneider, C.L., Ruppel, E.G., Hecker, R.J., and Hogaboam, G.J. 1982. Effect of soil deposition in crowns on development of Rhizoctonia root rot in sugarbeet. Plant Dis. 66:408-410.

Sneh, B., Burpee, L., and Ogoshi, A. 1991. Identification of *Rhizoctonia* species. American Phytopathological Society Press, MN, USA.

Stachler, J.M., Carlson, A.L., Luecke, J.L., Boetel, M.A., and Khan, M.F.R. 2009. Survey of weed control and production practices on sugarbeet in Minnesota and eastern North Dakota. SBREB. 40:39-60. Available at http://www.sbreb.org/research/weed/weed09/SurveyWeedControl2009.pdf. (Verified 11 Mar. 2015).

Stammler, G., Brix, H. D., Nave, B., Gold, R., and Schoefl, U. 2008. Studies on the biological performance of boscalid and its mode of action. In V. H. W. Dehne, H. B. Desing, U. Gisi, K. H. Kuck, P. E. Russel, & H. Lyr, (Eds), *Modern Fungicides and Antifungal Compounds* (pp. 45-51). DPG-Verlag, Braunschweig, Germany.

Stodart, B.J., Harvey, P.R., Neate, S.M., Melanson, D.L., and Scott, E.S. 2007. Genetic variation and pathogenicity of anastomosis group 2 isolates of *Rhizoctonia solani* in Australia. Mycological Res. 111:891-900.

Stump, W.L., Franc, G.D., Harveson, R.M., and Wilson, R.G. 2004. Strobilurin fungicide timing for Rhizoctonia root and crown rot suppression in sugarbeet. JSBR. 41:17-37.

Sumner, D.R. 1999. Rhizoctonia crown and brace root rot. In D.G. White (Ed), *Compendium of Corn Diseases* (pp. 12-13). The American Phytopathological Society, St. Paul, MN, USA.

Sumner, D.R., and Bell D.K. 1982. Root diseases induced in corn by *Rhizoctonia solani* and *Rhizoctonia zeae*. Phytopathology 72:86-91.

USDA-ERS. 2014. Sugar and sweeteners yearbook tables. Available at http://www.ers.usda.gov/datafiles/Sugar_and_Sweeteners_Yearbook_Tables/US_Sugar_Supply_and_Use/TABLE14.XLS. (Verified 11 Mar. 2015).

Vilgalys, R., and Cubeta, M.A. 1994. Molecular systematics and population biology of Rhizoctonia. Annual Review Phytopathology 32:135-155.

Webb, K.M. Harveson, R.M. and West, M.S. 2015. Evaluation of *Rhizoctonia zeae* as a potential biological control option for fungal root diseases of sugar beet. Ann. Appl. Biol. DOI: 10.1111/aab.12210.

Whitney, E.D., and Duffus, J.E. 1986. *Compendium of Beet Diseases and Insects*. The American Phytopathological Society, St. Paul, MN, USA.

Windels, C.E. 1988. Seedling and root rot disease of sugarbeet. SBREB. 19:125-132. Available at http://www.sbreb.org/research/plant/plant88/88p125.htm. (Verified 11 Mar. 2015).

Windels, C.E., and Brantner, J.R. 2002. Timing applications of quadris for control of Rhizoctonia on sugarbeet. SBREB 33:182-195. Available at http://www.sbreb.org/research/plant/plant02/27quadris.htm. (Verified 11 Mar. 2015)

Windels, C. E., and Brantner, J. R. 2004. Previous crop influences Rhizoctonia on sugarbeet. SBREB. 35:227- 231. Available at http://www.sbreb.org/04/Pathology%2004/Pathology9.pdf. (Verified 11 Mar. 2015)

Windels, C.E., and Brantner, J.R. 2005. Early-season application of azoxystrobin to sugarbeet for control of *Rhizoctonia solani* AG 4 and AG 2-2. JSBR. 42:1-16.

Windels, C.E., and Brantner, J.R. 2006. Rhizoctonia inoculum and rotation crop effects on a following sugarbeet crop. SBREB 37:182-191. Available at http://www.sbreb.org/research /plant/plant06/Rhizoctonia.pdf. (Verified 11 Mar. 2015).

Windels, C.E., and J.R. Brantner. 2006. Rhizoctonia inoculum and rotation effects on a following sugarbeet crop. SBREB. 37:182-191. Available at http://www.sbreb.org/research/plant /plant06/Rhizoctonia.pdf. (Verified 11 Mar. 2015).

Windels, C.E. and Brantner, J.R. 2009. Band-applied fungicides to control Rhizoctonia crown and root rot. SBREB. 37:182-191. Available at http://www.sbreb.org/research/plant/plant09 /BandAppliedFungicide2009.pdf. (Verified 11 Mar. 2015).

Windels, C.E., Jacobsen, B.J., and Harveson, R.M. 2009. Rhizoctonia root and crown rot. In R.M. Harveson, L.E. Hanson, and G.L. Hein (Eds.), *Compendium of Beet Diseases and Pests* (pp. 33-36). The American Phytopathological Society Press, St. Paul, MN, USA.

Windels, C.E., Kuznia, R.A., and Call, J. 1997. Characterization and pathogenicity of *Thanatephorus cucumeris* form sugarbeet in Minnesota. Plant Dis. 81:245-249.

Windels, C.E. and Nabben, D.J. 1989. Characterization and pathogenicity of anastomosis groups of *Rhizoctonia solani* isolated from *Beta vulgaris*. Phytopathology 79:83-88.

Yanase, Y., Yoshikawa, Y., Kishi, J., and Katsuta, H. 2007. The history of complex II inhibitors and the discovery of penthiopyrad. In H. Ohkawa, H. Miyagawa, and P.W. Lee (Eds.), *Pesticide Chemistry: Crop Protection, Public Health, Environmental Safety* (pp. 295-303). Wiley, New Jersey, NY, USA.

CHAPTER 2. EFFECT OF PENTHIOPYRAD AT DIFFERENT RATES AND USING DIFFERENT APPLICATION METHODOLOGIES ON *RHIZOCTONIA SOLANI* ON SUGARBEET

Introduction

Sugarbeet (*Beta vulgaris* L.) is grown in 50 countries as a primary source of sucrose and provides 20% of world's sugar production (FAO, 2014). The United States is one of leading sugarbeet producers around the world with ten producing states including California, Colorado, Idaho, Michigan, Minnesota, Montana, Nebraska, North Dakota, Oregon, and Wyoming. In 2014, Minnesota and North Dakota contributed 47% of the nation's sugarbeet production (USDA-ERS, 2014), which results in $4 billion worth of total economic activities.

Rhizoctonia solani Kühn (Teleomorph: *Thanatephorus cucumeris* (A.B. Frank) Donk), is considered to be the most serious pathogen in sugarbeet production of Minnesota and North Dakota (Stachler et al., 2009). *Rhizoctonia solani* leads to yield loss and reduced sucrose content of sugarbeet, and entire field can be destroyed if not managed (Khan et al., 2010; Windels and Brantner, 2005). This fungus is composed of different genetically isolated populations recognized as anastomosis groups (AGs) (Ogoshi, 1987). *Rhizoctonia solani* AG 2-2 IIIB and IV are the major causal agents found on sugarbeet and are widely distributed in the Red River Valley of North Dakota and Minnesota (Brantner and Windels, 2007). The pathogen causes damping-off of seedlings and crown and root rot of older plants. The optimum soil temperature for infection by *R. solani* ranges from 20°C to 30°C (Leach, 1986). High soil moisture increases disease severity (Bolton et al., 2010).

Agronomic practices, resistant cultivars, and fungicide applications are used together to protect sugarbeet from *R. solani*. Agronomic practices, including early spring planting in cool soils

(Khan et al., 2008; Windels, 1988), crop rotation with non-host crops such as barley and wheat (Windels and Brantner, 2006), and good field drainage may help reduce *R. solani* infection on sugarbeet. For many years, Rhizoctonia-resistant cultivars were not widely used because of their lower potential yield compared to susceptible and commercial cultivars (Panella and Ruppel, 1996). Growers typically use high yielding cultivars which were more susceptible to *R. solani* and rely on fungicides to protect sugarbeet field. Azoxystrobin (Quadris, Sygenta), a quinone outside inhibitor (QoI), was labeled for use on sugarbeet in 1999. QoI fungicides inhibit mitochondrial respiration in fungi by binding to the quinol site of the cytochrome bc1 complex, blocking electron transfer and halting ATP synthesis. Azoxystrobin effectively controls *R. solani* on sugarbeet when it is applied in a timely manner, that is, before infection takes place (Khan et al., 2010; Kiewnick et al., 2001; Windels and Brantner, 2005). Azoxystrobin was one of the most widely used fungicides in Minnesota and North Dakota for controlling *R. solani* on sugarbeet (Carlson et al., 2012). Around 2009, growers started using another QoI fungicide, pyraclostrobin (Headline, BASF), for managing *R. solani* on sugarbeet. The widespread and continuous use of one mode of action fungicide to control a pathogen is not recommended since this can result in the development of fungicide resistant fungal populations and fungicide failures. The QoI fungicides, because of their specific single site mode of action, are considered high risk for fungicide resistance.

Rhizoctonia solani has a sexual and asexual stage. However, the sexual stage is rarely found in sugarbeet and is not considered to play a role in disease development, the disease cycle or management. The asexual stage of *R. solani* is found in soils worldwide and cause diseases of many crops. Fungicides have been widely used for many years to control this pathogen in different patho-systems. Because of the monocyclic nature of the asexual stage of the pathogen, it was not considered to be amenable for developing resistance to fungicides. However, in 2012, Olaya et al.

reported the resistance of *R. solani* 1-IA on rice to azoxystrobin in Louisiana (Olaya et al., 2012). As a result it would be useful to have fungicides with different modes of action that can effectively manage *R. solani* and be used in rotation with QoI fungicides.

Penthiopyrad (Vertisan, Dupont), a succinate dehydrogenase inhibitor (SDHI), is a broad-spectrum fungicide that has antifungal activities against rust, rhizoctonia, and ascomycete diseases (Avenot and Michailides, 2010; Yanase et al., 2007). It would be useful to know whether penthiopyrad is able to prevent *R. solani* without being phytotoxic to sugarbeet, so that it can be considered as a rotating chemical for QoI fungicides. The objective of this greenhouse study was to evaluate the efficacy of penthiopyrad for managing *R. solani* on sugarbeet using different application methodologies.

Materials and methods

Research was conducted in a greenhouse at North Dakota State University, Fargo, ND, USA. Sunshine mix 1 peat (Sun Gro Horticulture Ltd.; Alberta, Canada) was filled into plastic trays (T. O. Plastics Inc.; Clearwater, MN, USA) measuring 27 x 13 x 13 cm and pots measuring 10 x 10 x 12 cm. Crystal 539RR, a cultivar susceptible to *R. solani* (Niehaus, 2011) was used in this research. *R. solani* AG 2-2 IIIB (obtained from Dr. Carol Windels, University of Minnesota, Crookston, MN, USA) was grown on sterilized barley grains for inoculum production as described by Noor and Khan (2014).

Three experiments were conducted to evaluate the efficacy of penthiopyrad (Vertisan, 20.6%, Dupont), compared with azoxystrobin (Quadris, 22.9%, Syngenta) which is considered as the industry's standard, at controlling *R. solani* on sugarbeet using in-furrow (experiment 1), band (experiment 2), and soil drench (experiment 3) applications. Penthiopyrad was used at 550, 420, 280, and 210 g a.i. ha^{-1} and azoxystrobin was applied at 167 g a.i. ha^{-1}. In experiment 1 (in-furrow

application), a furrow 2.5-cm soil deep was made in the center of each tray into which 10 seeds were spaced evenly. Fungicides were then applied directly over the seeds, followed by placing one *R. solani*-infested barley grain 1-cm away from each seed (Noor and Khan, 2014). The seeds and inoculum were covered with the Sunshine mix peat which was then compacted over the seeds. In experiment 2 (band application), three seeds were planted 2-cm deep in each pot and thinned at the two-leaf stage to allow one vigorous plant per pot. When these sugarbeet plants were at the 4-leaf stage, fungicides were applied in an 18-cm band targeting the leaves and especially the soil, followed by placing one infested barley grain 2-cm deep and 1-cm away from each plant. The in-furrow and 18-cm band applications were conducted using a spraying system (De Vries Manufacturing; Hollandaise, MN, USA) calibrated to deliver 47 L ha^{-1} solution at 138 kPal through a single flat fan nozzle (4001E). In experiment 3 (soil drench application), 4-leaf stage sugarbeet plants were used. The treatments (1 ml of fungicide solution) were injected with a syringe (HSW Norm-Ject; Dudley, MA, USA) close to the soil-hypocotyl interface and 1-cm soil deep followed by inoculation as described above. The amount of fungicide solution for each plant was determined based on the active ingredient per hectare divided by the total number of sugarbeet plants in the area (98,800 plants ha^{-1}). The positive control was inoculated with *R. solani*-infested barley grains while the negative control was inoculated with sterilized barley grains without *R. solani*. The greenhouse conditions were set to allow for a 12-h photoperiod and temperature was maintained at 22 ± 2°C (Argus Control Systems Ltd.; British Columbia, Canada). Sugarbeet plants were watered daily to maintain adequate moisture favorable for plant growth and disease development.

Experiment 1 was repeated three times as a randomized complete block design (RCBD) with four replicates. Fungicide-treated seedlings and plants were observed for phytotoxicity

symptoms such as stunting, leaf curling, and misshapen leaves. Sugarbeet survivors were counted at 28 days after inoculation (DAI), and their roots were carefully removed from trays, washed under running tap water, and evaluated for root rot symptoms present on the tap root. Levene's test was performed for homogeneity of variances across three repeats. Analysis of variance (ANOVA) for plant survival was conducted using the SAS general linear models (Proc GLM) procedure (Version 9.3, SAS Institute Inc.; Cary, NC, USA). Treatment means were separated by calculating Fisher's Least Significant Difference at α=0.05 confidence level.

Experiment 2 and 3 were each repeated twice in a completely randomized design (CRD) with six replicates. At 21 DAI, sugarbeet plants were carefully removed from pots and their roots were washed under tap water. Root rot symptoms were evaluated using a 0 to 7 scale: 0 (no disease), 1 (crown area slightly scurfy), 2 (<5% infection), 3 (6-25% infection), 4 (26-50% infection), 5 (51-75% infection), 6 (>75% infection), and 7 (the root completely deteriorated or dead plant) (Windels and Nabben-Schindler, 1996). The disease scales were analyzed by non-parametric analysis where mean rank was calculated by running the SAS procedures of Proc Rank and Proc Mixed. The relative effect of disease severity for each treatment with its confidence interval was calculated using LD-CI macro (Shah and Madden, 2004).

Results

In experiment 1 (in-furrow application), there was no significant difference between repeats based on Levene's test, so the plant survival data were combined. Difference in plant survival was significant among treatments at $P \leq 0.001$ (Table 2.1). There were only 4% survivors in the positive control which was significantly lower than the 86% in the negative control (Figure 2). The high mortality in the positive control indicated that the inoculum was effective at killing seedlings. Penthiopyrad at all rates resulted in significantly higher percentage survival than the

positive control, and except for the lowest rate (210 g a.i. ha^{-1}), resulted in similar percentage survival as the negative control. There were no significant differences in percentage survivals among the different rates of penthiopyrad. The standard azoxystrobin in-furrow application resulted in percentage survival that was similar to the negative control. All the penthiopyrad rates, except the lowest (210 g a.i. ha^{-1}), resulted in percentage survival that was not significantly different from that obtained by the standard azoxystrobin treatment. Visual symptoms of plant injury such as stunting and leaf curl were not observed in any of the fungicide treatments. The plants from the negative control and fungicide treatments had clean roots without any typical *R. solani* symptoms.

In experiment 2 (band application experiment), all the surviving sugarbeet plants were healthy in the negative control but there was 91% mortality in the positive control (Table 2.2). All rates of penthiopyrad applied as a band were not effective at controlling *R. solani* and resulted in similar mean disease severity as the positive control. Azoxystrobin applied as a band application did not provide complete disease management since some roots had minor infections compared with the negative control. However, azoxystrobin applied in a band resulted in significantly lower relative effect of disease severity compared with the positive control and the different rates of penthiopyrad.

In experiment 3 (soil drench application), the positive control resulted in death of the plants whereas the negative control resulted in healthy surviving plants (Table 2.3). Penthiopyrad at all rates and azoxystrobin provided protection against *R. solani* and prevented infection, resulting in disease control similar to the negative control. No phytotoxic symptoms were observed on sugarbeet treated with azoxystrobin and penthiopyrad in the soil drench application.

Table 2.1: Combined analysis of variance (ANOVA) for the number of plant survivals from sugarbeet seeds treated with penthiopyrad and azoxystrobin fungicides applied in-furrow, followed by inoculation with *R. solani* AG 2-2 IIIB.

Sources of variation	DF	*P*-value
Repeats	2	0.480
Treatments	5	<0.001**
Treatments×Experiments	10	0.420
Error	45	-
Total	71	-

**Indicates significance at $P \leq 0.001$ level of confidence.

Table 2.2: Effect of penthiopyrad and azoxystrobin fungicides applied in an 18-cm band application on disease severity of 4-leaf stage sugarbeet inoculated with R. solani AG 2-2 IIIB and evaluated at 21 days after inoculation (DAI).

Treatment	MDS[a]	Mean rank	Mortality (%)	RED S[b]	95% CI of the RE[c]	
					Lower limit	Upper limit
550 g a.i. ha^{-1}[d] Penthiopyrad	7	26	58.3	0.62	0.53	0.7
420 g a.i. ha^{-1} Penthiopyrad	7	25.1	58.3	0.59	0.5	0.69
280 g a.i. ha^{-1} Penthiopyrad	7	25.5	66.7	0.61	0.5	0.7
210 g a.i. ha^{-1} Penthiopyrad	7	27.6	75	0.66	0.56	0.74
167 g a.i. ha^{-1}[e] Azoxystrobin	0	7.3	0	0.17	0.15	0.21
Negative control	0	5	0	0.12	0.1	0.14
Positive control	7	30.3	91.7	0.72	0.66	0.78

[a] Median of disease scales based on a 0-7 scale: 0 (no disease), 1 (crown area slightly scurfy), 2 (<5% infection), 3 (6-25% infection), 4 (26-50% infection), 5 (51-75% infection), 6 (>75% infection), and 7 (the root completely deteriorated or dead plant).
[b] Relative effect of disease severity.
[c] 95% confidence intervals of the relative effect.
[d] Penthiopyrad at 550, 420, 280, and 210 g a.i. ha^{-1} was applied in an 18-cm band.
[e] Azoxystrobin at 167 g a.i. ha^{-1} was applied in an 18-cm band.

Table 2.3: Effect of penthiopyrad and azoxystrobin fungicides used as a soil drench by injection close to the soil-root interphase on disease severity of 4-leaf stage sugarbeet inoculated with *R. solani* AG 2-2 IIIB and evaluated at 21 DAI.

Treatment	MDS	Mean rank	Mortality (%)	REDS	95% CI of the RE	
					Lower limit	Upper limit
550 g a.i. ha^{-1} Penthiopyrad	0	20.0	0	0.48	0.40	0.56
420 g a.i. ha^{-1} Penthiopyrad	0	18.5	0	0.44	0.38	0.51
280 g a.i. ha^{-1} Penthiopyrad	0	18.5	0	0.43	0.38	0.50
210 g a.i. ha^{-1} Penthiopyrad	0	17.0	0	0.40	0.38	0.43
167 g a.i. ha^{-1} Azoxystrobin	0	17.0	0	0.40	0.38	0.43
Negative control	0	17.0	0	0.40	0.38	0.43
Positive control[a]	7	40.0	100	0.93	-	-

[a]Positive control had the same and highest exclusive scales, so its relative effect was constant.

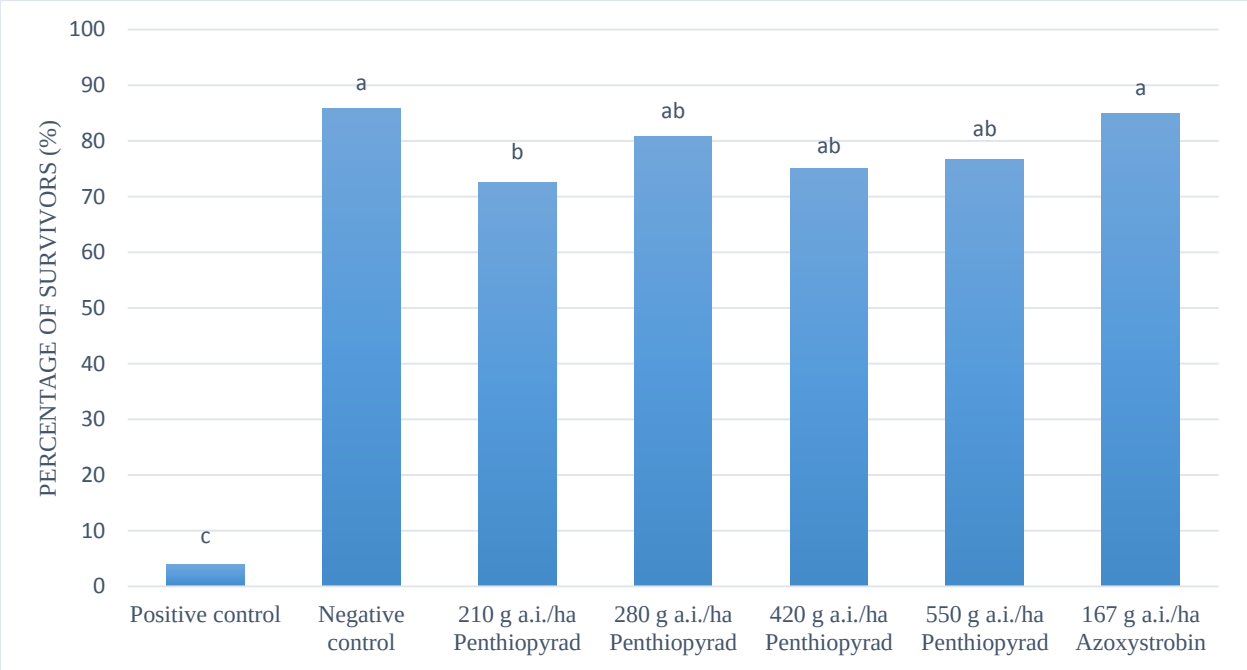


Figure 2.1: Percentage of sugarbeet survivors after treatment with penthiopyrad or azoxystrobin fungicides, followed by inoculation with *R. solani* AG 2-2 IIIB. Penthiopyrad (Vertisan, 20.6%, DuPont) was applied in-furrow at 210, 280, 420, and 550 g a.i. ha^{-1}. Azoxystrobin (Quadris, 22.9%, Syngenta) was applied in-furrow at 167 a.i. ha^{-1}. Treatments with the same letter were not significantly different at $P \leq 0.05$ level of confidence.

Discussion

The SDHIs were first used in agriculture since the 1960s with a narrow spectrum of activity. Since 2003, newer molecules including penthiopyrad were developed which are potent against a wider spectrum of pathogens. Penthiopyrad, developed by Mitsubishi Kasei Corporation, is a novel SDHI fungicide which displays a broad spectrum of antifungal activity against ascomycetes and basidiomycetes pathogens including rhizoctonia diseases (Yanase et al., 2007). Compared to QoIs, the SDHI fungicides also interrupt fungal respiration but act in the succinate dehydrogenase complex by blocking the ubiquinone-binding sites in mitochondrial complex II (Avenot and Michailides, 2010). Due to its unique mode of action, penthiopyrad was classified into FRAC (Fungicide Resistance Action Committee) group 7 and has no known cross resistance with azoxystrobin in FRAC group 11. In this study, penthiopyrad was evaluated as a 20.6 EC formulation product of DuPont using in-furrow and band application methodologies as recommended on the label, as well as the more directed soil drench application for management of the soil-borne *R. solani* on sugarbeet.

In this study, all rates of penthiopyrad except the lowest rate applied in-furrow provided similar levels of efficacy at controlling *R. solani* compared with azoxystrobin. Neher and Keeth (2012) reported that penthiopyrad and azoxystrobin applied in-furrow resulted in similar seedling stand, disease index, and disease incidence in sugarbeet in a field study. The recommended labeled rate for this product in-furrow is 30 fluid oz per acre which is equivalent to 420 g a.i ha^{-1}. This study showed that in-furrow application of azoxystrobin resulted in a comparable seedling population to the negative control. The high efficacy of azoxystrobin applied in-furrow against *R. solani* has been confirmed in field and greenhouse studies (Brantner and Windels, 2010; Noor and

Khan, 2014; Windels and Brantner, 2005). In North Dakota and Minnesota, azoxystrobin has been widely used in-furrow to control *R. solani* in sugarbeet field. It will be useful for growers to have penthiopyrad, another mode of action that can be used in-furrow in alternating years with azoxystrobin for controlling *R. solani* and be used as a fungicide resistance management strategy.

Band application of fungicides which target the soil around the roots is typically required to control rhizoctonia root rot in fields with a history of the disease since root rot occurs when the pathogen initiates infection to older plants (Stump et al., 2004). In band applications, some of the fungicides will fell on the leaves. Since *R. solani* is a soil-borne fungus, fungicide applied to the leaves needs to be translocated to the roots to provide protection. However, penthiopyrad and azoxystrobin are not known to redistribute themselves downward from the leaves to plant roots (Bartlett et al., 2001). In this study, the band application of penthiopyrad was ineffective at controlling *R. solani*. Azoxystrobin provided significantly better disease control than penthiopyrad, but there were a few plants with some minor infections which resulted in a higher relative effective of disease severity compared with the negative control. Poindexter and Wenzel (2013) also reported that band application of penthiopyrad was not effective at controlling *R. solani* while azoxystrobin was effective in field trials in Michigan. Both fungicides were applied to the soil surface and plant foliage and were washed downwards with irrigation water to get into contact with roots and pathogen. The in-furrow application, where both fungicides were applied directly over the seeds and close to the pathogen prevented infection. Penthiopyrad and azoxystrobin typically inhibit spore germination of pathogens which they control (Bertelsen et al., 2001, Yanase et al., 2013). Since only azoxystrobin provided control against *R. solani*, it is possible that the fungicides had different rates of movement through the potting mix and azoxystrobin was transported faster and close enough to the roots and pathogen for it to be effective, whereas

penthiopyrad was probably bound to the potting mix and was not transported at a high enough concentration in time to prevent germination of the sclerotia to achieve disease control.

Soil drench facilitates direct contact of the fungicidal solution with the hypocotyl and root and therefore is useful for controlling soil-borne diseases where this application method is possible. Soil drench will typically require a high volume of solution to target the base of the plant and allow deep penetration to the root area. Soil drench is not practiced by sugarbeet growers in North Dakota and Minnesota. The soil drench application method was used in this study to determine whether fungicides will prevent infection when in close proximity to the roots that need protection and the pathogen. Penthiopyrad, when used as a drench, provided excellent disease control similar to the industry standard, azoxystrobin, which were not significantly different from the negative control. This result is consistent with Meyer and Hausbeck (2013), who showed that fungicide drenches were more effective at controlling *Phytophthora capsici* Leonian on summer squash than foliar applications since this method allows the fungicide to be in closer proximity or direct contact the plant roots which are targeted by soil-borne pathogens.

This study demonstrated that penthiopyrad applied at 280, 420, and 550 g a.i ha^{-1} provided effective control of *R. solani* when the fungicide was in close proximity to the seeds or the roots and the pathogen before the pathogen becomes infective. The level of disease control provided by penthiopyrad at the higher rates was similar to that provided by azoxystrobin, the industry standard. Rhizoctonia has been listed as the number one problem by growers since 2009 and QoI fungicides, mainly azoxystrobin, has been used in-furrow to control *R. solani*. Olaya et al. (2012) have reported that *R. solani* can develop resistance to QoI fungicides and lead to field failures of these fungicides. Arabiat (2015) reported that some *R. solani* isolates collected from sugarbeet fields have relatively high EC_{50} values against QoI fungicides. Penthiopyrad was labeled for use

as an in-furrow and band-application treatment for sugarbeet in 2012. Unfortunately, this fungicide is not available for sale commercially as of 2015. It would be useful to have penthiopyrad so that it could be rotated with azoxystrobin for in-furrow applications not only to control *R. solani* but also to help prevent the buildup of populations resistant to QoI fungicides which is possible with continuous use of only one mode of action fungicide.

Literature cited

Arabiat, A. 2015. Sensitivity of *Rhizoctonia solani* and *Aphanomyces cochlioides* to fungicides, and fitness of tetraconazole-resistant isolates of *Cercospora beticola* after exposure to different temperature regimes (PhD dissertation). North Dakota state Univ. Institutional Repository, Fargo, ND. T18:38:57Z.

Avenot, H.F., and Michailides, T.J. 2010. Progress in understanding molecular mechanisms and evolution of resistance to succinate dehydrogenase inhibiting fungicides in phytopathogenic fungi. Crop Prot. 29:643-651.

Bartlett, D.W., Clough, J.M., Godfrey, C.R., Godwin, J.R., Hall, A.A., Heaney, S.P., and Maund, S.J. 2001. Understanding the strobilurin fungicides. Pesticide Outlook 12:143-148.

Bertelsen, J.R., De Neergaard, E., and Smedegaard-Petersen, V. 2001. Fungicidal effects of azoxystrobin and epoxiconazole on phyllosphere fungi, senescence and yield of winter wheat. Plant Pathol. 50:190–205.

Bolton, M.D., Panella, L., Campbell, L., and Khan, M.F.R. 2010. Temperature, moisture, and fungicide effects in managing Rhizoctonia root and crown rot of sugarbeet. Phytopathology 100:689-697.

Brantner, J.R., and Windels, C.E. 2007. Distribution of *Rhizoctonia solani* AG 2-2 intraspecific groups in the Red River Valley and southern Minnesota. SBREB. 38:242–246. Available at http://www.sbreb.org/research/plant/plant07/08DistributionOfRhizocRRV.pdf. (Verified 11 Mar. 2015).

Carlson, A.L., Khan, M.F.R., Stachler, J.M., and Boetel, M.A. 2012. Survey of fungicide use in sugarbeet in Minnesota and eastern North Dakota in 2012. SBREB. 42:181-182. Available At http://www.sbreb.org/research/plant/plant11/CarlsonSurveyFungicide2012.pdf. (Verified 11 Mar. 2015).

FAO. 2014. FAO Statistical Yearbook 2014. Available at http://www.fao.org/3/a-i3621e.pdf. (Accessed 11 Mar. 2015).

Khan, M.F.R., Khan, J., and Bradley, C.A. 2010. Effect of azoxystrobin applications based on soil temperature on Rhizoctonia root and crown rot of sugarbeet. Int. Sugar J. 112:557-560.

Khan, M.F.R., Qandah, I., and Bolton, M.D. 2008. The effect of temperature on Rhizoctonia disease development and fungicide efficacy in controlling Rhizoctonia root rot on sugarbeet. SBREB. 39:252-254. Available at http://www.sbreb.org/research/plant/plant08/TemperatureRhizoctonia.pdf. (Accessed 11 Mar. 2015).

Kiewnick, S., Jacobsen, B.J., Braun-Kiewnick, A., Eckhoff, J.L.A., and Bergman, J.W. 2001. Integrated control of Rhizoctonia crown and root rot of sugarbeet with fungicides and antagonisitic bacteria. Plant Dis. 85:718-722.

Leach, L.D. 1986. Seedling diseases. In: E.D. Whitney and J.E. Duffus (Eds.), Compendium of beet diseases and insects (pp. 4-8). The American Phytopathological Society Press, St. Paul, MN, USA.

Meyer, M.D., and Hausbeck, M.K. 2013. Using soil-applied fungicides to manage Phytophthora crown and root rot on summer squash. Plant Dis. 97:107-112.

Neher, O.T., and Keeth T. 2013. Comparison of in-furrow applied fungicides for control of Rhizoctonia crown and root rot, 2012. Plant Dis. Manag. Rep. 7:FC073.

Niehaus, W.S. 2011. Results of American Crystal's 2011 official coded variety trails. SBREB. 42:276-346. Available at http://www.sbreb.org/research/sugar/sugar11/ResultsACSCNDSUReport.pdf. (Verified 11 Mar. 2015).

Noor, A., and Khan, M.F.R. 2014. Efficacy and safety of mixing azoxystrobin and starter fertilizers for controlling *Rhizoctonia solani* in sugarbeet. Phytoparasitica 43:51-55.

Ogoshi, A. 1987. Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizcotonia solani* Kühn. Annu. Rev. Phytopathol. 25:125-143.

Olaya, G., Buitrago, C., Pearsaul, D., Sierotzki, H., and Tally, A. 2012. Detection of resistance to QoI fungicides in *Rhizoctonia solani* isolates from rice. Phytopathology 102: S4.88.

Panella, L., and Ruppel, E.G. 1996. Availability of germplasm for resistance against *Rhizoctonia* Spp. In B. Sneh, S. Jabaji-Hare, S. Neate, & G. Dijst (Eds.), *Rhizoctonia species: Taxonomy, molecular biology, ecology, pathology and disease control* (pp. 515-527). Kluwer Academic publishers, Dordrecht, Netherlands.

Poindexter, S.S., and Wenzel, T.J. 2013. Quadris (azoxystrobin) and vertisan (penthiopyrad) fungicide efficacy comparisons on natural Rhizoctonia infections in sugarbeets. In ASSBT proceedings from the 37th Biennial Meeting. Anaheim, CA, 27 Feb.-3 Mar. 2013. Available at http://assbt-proceedings.org/ASSBT2013Proceedings/SecC/QuadrisAndVertisan.pdf. (Verified 11 Mar. 2015)

Shah, D.A., and Madden, L.V. 2004. Nonparametric analysis of ordinal data in designed factorial experiments. Phytopathology 94:33-43.

Stachler, J.M., Carlson, A.L., Luecke, J.L., Boetel, M.A., and Khan, M.F.R. 2009. Survey of weed control and production practices on sugarbeet in Minnesota and eastern North Dakota.

SBREB. 40:39-60. Available at http://www.sbreb.org/research/weed/weed09/SurveyWeedControl2009.pdf. (Verified 11 Mar. 2015).

Stump, W.L., Franc, G.D., Harveson, R.M., and Wilson, R.G. 2004. Strobilurin fungicide timing for Rhizoctonia root and crown rot suppression in sugarbeet. JSBR. 41:17-37.

USDA-ERS. 2014. Sugar and sweeteners yearbook tables. Available at http://www.ers.usda.gov/datafiles/Sugar_and_Sweeteners_Yearbook_Tables/US_Sugar_Supply_and_Use/TABLE14.XLS. (Verified 11 Mar. 2015).

Windels, C.E., and Brantner, J.R. 2005. Early-season application of azoxystrobin to sugarbeet for control of *Rhizoctonia solani* AG 4 and AG 2-2. JSBR. 42:1-16.

Windels, C.E., and Brantner, J.R. 2006. Rhizoctonia inoculum and rotation crop effects on a following sugarbeet crop. SBREB 37:182-191. Available at http://www.sbreb.org/research/plant/plant06/Rhizoctonia.pdf. (Verified 11 Mar. 2015).

Windels, C.E., and Nabben-Schindler, D.J. 1996. Limitations of a greenhouse bioassay for determining potential of Aphanomyces root rot in sugarbeet fields. JSBR. 33:1-13.

Windels, C.E. 1988. Seedling and root rot disease of sugarbeet. SBREB. 19:125-132. Available at http://www.sbreb.org/research/plant/plant88/88p125.htm. (Verified 11 Mar. 2015)

Yanase, Y., Katsuta, H., Tomiya, K., Enomoto, M., and Sakamoto, O. 2013. Development of a novel fungicide, penthiopyrad. J. Pestic. Sci. 38:167–168.

Yanase, Y., Yoshikawa, Y., Kishi, J., and Katsuta, H. 2007. The history of complex II inhibitors and the discovery of penthiopyrad. In H. Ohkawa, H. Miyagawa, and P.W. Lee (Eds.), Pesticide Chemistry: Crop Protection, Public Health, Environmental Safety (pp. 295-303). Wiley, New Jersey, NY, USA.

CHAPTER 3. EFFICACY OF PENTHIOPYRAD AS A SEED TREATMENT ON SUGARBEET AT CONTROLLING *RHIZOCTONIA SOLANI*

Introduction

Sugarbeet is one of the two resources of sucrose and represents 20% of the world's sugar production (FAO, 2014). It is one of the leading raw materials for sugar production in the United States. Ten states in the United States produced 31.3 million tons of sugarbeet from 1.14 million acres in 2014. The greatest volume of production occurs in Minnesota and North Dakota and their growers contribute 47% of the nation's sugarbeet production in 2014 (USDA-ERS 2014).

Sugarbeet is susceptible to numerous diseases such as Cercospora leaf spot (*Cercospora beticola* Saccardo), Aphanomyces root rot (*Aphanomyces cochlioides* Drechsler), and Rhizomania (beet necrotic yellow vein virus transmitted by *Polymyxa betae* Keskin). However, a soil-pathogen *Rhizoctonia solani* Kühn (Teleomorph *Thanatephorus cucumeris* (A.B. Frank) Donk) was reported as the most serious problem by sugar beet growers in Minnesota and North Dakota (Stachler et al., 2009). The two subgroups *R. solani* AG 2-2 IIIB and IV were the most virulent and widely distributed in sugarbeet producing area in Minnesota and North Dakota (Brantner and Windels, 2007). The pathogen not only causes damping-off of sugar beet seedlings but also crown and root rot on older plants, leading to significant yield loss when environmental conditions are wet and warm (Khan et al., 2010; Windels and Brantner, 2005).

Fungicide application is the major method used for controlling *R. solani* in sugarbeet fields. Azoxystrobin (Quadris, Syngenta), used as both in-furrow and foliar applications, provided effective control of *R. solani* (Windels and Brantner, 2005; Khan and Carlson, 2009). Azoxystrobin belongs to QoI fungicides within FRAC (Fungicide Resistance Action Committee)

group 11. QoI fungicides were considered as high risk for resistance development due to their single-site mode of action. Resistance of *R. solani* AG 1-IA to azoxystrobin was reported on rice in the United States, which has raised concerns about fungicide resistance management for this pathogen (Olaya et al., 2012). Moreover, Syngenta does not recommend the use of a starter fertilizer with azoxystrobin for in-furrow application in sugarbeet (Quadris label available at http://www.cdms.net/LDat/ld5QN008.pdf).

Seed treatment can be used to control soil-borne pathogens, which might be a safer method to apply fungicides than an in-furrow application, especially when fertilizer needs to be applied at the time of planting and the available planting time is very limited. The objective of this research was to compare the efficacy of penthiopyrad seed treatments with azoxystrobin for controlling *R. solani* on a Rhizoctonia susceptible and resistant sugarbeet cultivar.

Materials and methods

Research was conducted in a greenhouse at North Dakota State University in Fargo, ND, USA. Plastic trays measuring 27 x 13 x 13 cm (T. O. Plastics Inc.; Clearwater, MN, USA) were filled with Sunshine mix 1 peat (Sun Gro Horticulture Ltd.; Alberta, Canada). The isolates of *R. solani* AG 2-2 IIIB was obtained from Dr. Carol Windels (University of Minnesota, Crookston, MN). These cultures were used to inoculate sterilized barley grains for mass production as described by Kirk et al. (2008) with some slight modifications.

Sugarbeet cultivar 89RR10 (Betaseed, Inc.; Shakopee, MN, USA) was used as a Rhizoctonia resistant variety while sugarbeet cultivar 89RR50 (Betaseed, Inc.; Shakopee, MN, USA) was a Rhizoctonia susceptible variety (Niehaus, 2013). Furrows, 2.5-cm deep, were made in the middle of each tray into which ten evenly spaced sugarbeet seeds were planted. Penthiopyrad (Kabina ST, 40%, Mitsui, Japan) was used as a seed treatment at 5, 7, and 14 g a.i.unit^{-1} (100,000

seeds unit^{-1}) of seeds. Seed treatments were done by Betaseed, Inc., Tangent, OR, USA. Azoxystrobin (Quadris, 22.9%, Syngenta) was used at 167 g a.i.ha^{-1} and applied directly over the sugarbeet seeds in the in-furrow treatment, and directly over the seedlings and targeting the soil in the band (18-cm) application treatment. Azoxystrobin was applied using a spraying booth (De Vries Manufacturing; Hollandaise, MN, USA) calibrated to deliver 47 L ha^{-1} at 138 kPal through a single flat fan nozzle (4001E). There were 10 treatments for each sugarbeet cultivar. The treatments were as follows: penthiopyrad treated seeds at 5, 7, and 14 g a.i. unit^{-1} of seeds; penthiopyrad treated seeds at 5, 7, and 14 g a.i. unit^{-1} of seeds followed 14 days after planting with an 18-cm band application of azoxystrobin; azoxystrobin applied in-furrow directly over seeds with no penthiopyrad at planting; azoxystrobin applied in an 18-cm band 14 days after planting of seeds with no penthiopyrad; negative controls were inoculated with sterilized barley grain without *R. solani*; and positive controls were inoculated with *R. solani* infested barley grains. Inoculation was done using a stainless steel tweezer to place one Rhizoctonia-infested barley grain 1 cm to the side of each sugarbeet seed (Noor and Khan, 2014). After inoculation, seeds and inoculum were covered with Sunshine mix 1 peat soil. The conditions in the greenhouse were set to allow for a 12-h photoperiod and temperature was maintained at 22 ± 2°C (Argus Control Systems Ltd.; British Columbia, Canada). Trays were watered daily (3100 ml made on day 1 followed by about 300 ml daily per tray) to provide adequate moisture for plant growth and disease development. Plant survival was recorded by counting sugarbeet plant survivors on 10, 20, 30, and 45 DAI. Infected plants were collected to confirm the presence of *R. solani* on tap root. The experimental layout was a randomized complete block design (RCBD) with four replicates and the trial was repeated once under the same environmental conditions.

Plant survival data on 10, 20, 30, and 45 DAI were transformed into the area under the disease progress curve (AUDPC) for evaluating the effectiveness of treatments. AUDPC was calculated as:

$$\text{AUDPC}= \sum_{i=1}^{n-1}[(y_i + y_{i+1})/2](t_{i+1} - t_i)$$

where y_i= disease incidence at the i*th* observation, t_i= time (days) at the i*th* observation, and n = total number of observations (Shaner and Finney, 1977). Significance among contrast analysis was calculated at $\alpha \leq 0.05$ confidence level by comparing AUDPC using the SAS general linear models (Proc GLM) procedure (Version 9.3, SAS Institute Inc.; Cary, NC, USA).

Results

There was no significant difference in AUDPC on 10, 20, 30, and 45 DAI between the positive control of the resistant and susceptible cultivars, therefore, the data in this experiment were combined across the two cultivars. The negative controls had the greatest survivors (93.6%) which was very high relative to several greenhouse studies (Noor and Khan, 2014) (Table 3.1; Figure 3.1). At 10 DAI, the pathogen was already affecting the plants as measured by AUDPC. The positive control had a similar AUDPC as the treatment where no penthiopyrad was used but was to receive a band application of azoxystrobin at 14 DAI. These two treatments had significantly higher AUDPC compared to the penthiopyrad seed treatments and the azoxystrobin in-furrow application. The AUDPC for the different treatments at 20 DAI were similar to the results for the same treatments obtained at 10 DAI, including the treatments where azoxystrobin was band-applied at 14 DAI to treatments with penthiopyrad at 5, 7, and 14 g a.i unit^{-1} treated seeds. At 30 DAI, AUDPC increased. However, penthiopyrad seed treatments were still providing similar control as the standard azoxystrobin in-furrow treatment. At 45 DAI, all rates of penthiopyrad seed treatments resulted in significantly lower AUDPC than the positive control, but

there were some significant differences among the different penthiopyrad seed treatments. The 5 and 7 g a.i unit^{-1} penthiopyrad seed treatments had significantly higher AUDPC compared with the 14 g a.i. unit^{-1} rate. Disease control in the 5 and 14 g a.i unit^{-1} penthiopyrad seed treatments was not improved with the banded application of azoxystrobin on 14 DAI. However, the banded azoxystrobin did significantly improve disease control in the 7 g a.i unit^{-1} penthiopyrad seed treatment. The 14 g unit^{-1} penthiopyrad seed treatment, and the 7 g a.i. unit^{-1} penthiopyrad seed treatment enhanced with banded azoxystrobin 14 DAI resulted in similar AUDPC as azoxystrobin applied in-furrow (Figure 3.2).

Table 3.1: Contrast analysis of AUDPC between two classified groups of treatments on 10, 20, 30, and 45 DAI.

Group #1	Group #2	*P* > F			
		10 DAI[h]	20 DAI	30 DAI	45 DAI
Resistant cultivar[a]	Susceptible cultivar[b]	0.162	0.117	0.291	0.757
Negative control	Positive control	<0.001**	<0.001**	<0.001**	<0.001**
In-furrow	Negative control	0.038*	0.025*	0.029*	0.032*
In-furrow	Positive control	<0.001**	<0.001**	<0.001**	<0.001**
5g[c]	7g[d]	1.000	0.769	0.852	0.946
7g	14g[e]	0.465	0.529	0.730	0.026*
5g	14g	0.465	0.737	0.595	0.022*
5g	5g + foliar appl[f]	0.348	0.276	0.277	0.171
7g	7g + foliar appl	0.834	0.706	0.168	<0.001**
14g	14g + foliar appl	0.917	1.000	1.000	0.899
5g	In-furrow[g]	1.000	0.529	0.068	<0.001**
7g	In-furrow	1.000	0.737	0.101	<0.001**
14g	In-furrow	0.465	0.335	0.194	0.088
5g	Negative control	0.038*	0.004*	<0.001**	<0.001**
7g	Negative control	0.038*	0.011*	<0.001**	<0.001**
14g	Negative control	0.005*	0.002*	<0.001**	<0.001**
5g	Positive control	<0.001**	<0.001**	<0.001**	<0.001**
7g	Positive control	<0.001**	<0.001**	<0.001**	<0.001**
14g	Positive control	<0.001**	<0.001**	<0.001**	<0.001**
5g + foliar appl	In-furrow	0.348	0.645	0.457	0.009*
7g + foliar appl	In-furrow	0.834	0.967	0.790	0.516
14g + foliar appl	In-furrow	0.404	0.335	0.194	0.068
5g + foliar appl	Negative control	0.251	0.043*	0.004*	<.001**

Table 3.1: Contrast analysis of AUDPC between two classified groups of treatments on 10, 20, 30, and 45 DAI (continued).

Group #1	Group #2	*P* > F			
		10 DAI[h]	20 DAI	30 DAI	45 DAI
7g + foliar appl	Negative control	0.062	0.028*	0.015*	0.006*
14g + foliar appl	Negative control	0.004*	0.002*	<0.001**	<0.001**
5g + foliar appl	Positive control	<0.001**	<0.001**	<0.001**	<0.001**
7g + foliar appl	Positive control	<0.001**	<0.001**	<0.001**	<0.001**
14g + foliar appl	Positive control	<0.001**	<0.001**	<0.001**	<0.001**
foliar appl	In-furrow	<0.001**	<0.001**	<0.001**	<0.001**
foliar appl	5g	<0.001**	<0.001**	<0.001**	<0.001**
foliar appl	7g	<0.001**	<0.001**	<0.001**	<0.001**
foliar appl	14g	<0.001**	<0.001**	<0.001**	<0.001**
foliar appl	5g + foliar appl	<0.001**	<0.001**	<0.001**	<0.001**
foliar appl	7g + foliar app	<0.001**	<0.001**	<0.001**	<0.001**
foliar appl	14g + foliar appl	<0.001**	<0.001**	<0.001**	<0.001**
foliar appl	Negative control	<0.001**	<0.001**	<0.001**	<0.001**
foliar appl	Positive control	0.531	0.295	0.277	0.135

*Significantly different at $P \leq 0.05$.
**Significantly different at $P \leq 0.001$.
[a]The positive control where resistant seeds were planted.
[b]The positive control where susceptible seeds were planted.
[c]5g a.i. unit^{-1} penthiopyrad seed treatment.
[d]7g a.i. unit^{-1} penthiopyrad seed treatment.
[e]14g a.i. unit^{-1} penthiopyrad seed treatment.
[f]In-furrow application of azoxystrobin at 167 g a.i. ha^{-1}.
[g]Foliar application of azoxystrobin at 167 g a.i. ha^{-1}.
[h]Days of inoculation.

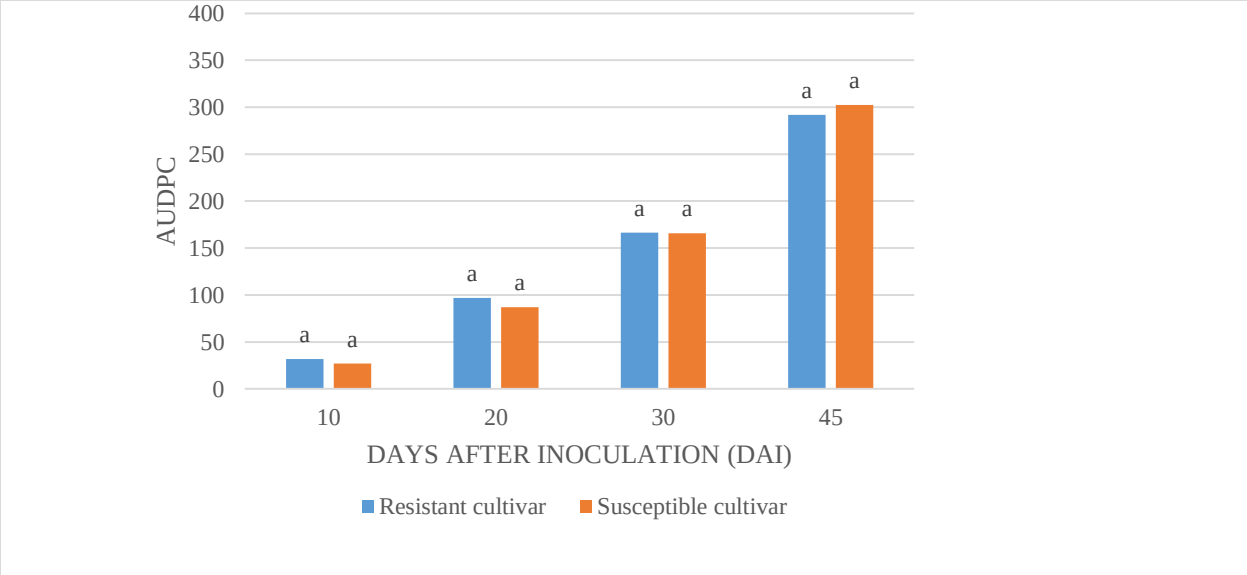


Figure 3.1: Effect of resistant and susceptible varieties on disease development of *R. solani* AG 2-2 IIIB from 10 DAI to 45 DAI. Resistant: *R. solani*-resistant cultivar 89RR10; Susceptible: *R. solani*-susceptible cultivar 89RR50. The treatments with the same number were not significantly different at $P \leq 0.05$ according to the contrast analysis of AUDPC between resistant and susceptible cultivars in the positive controls.

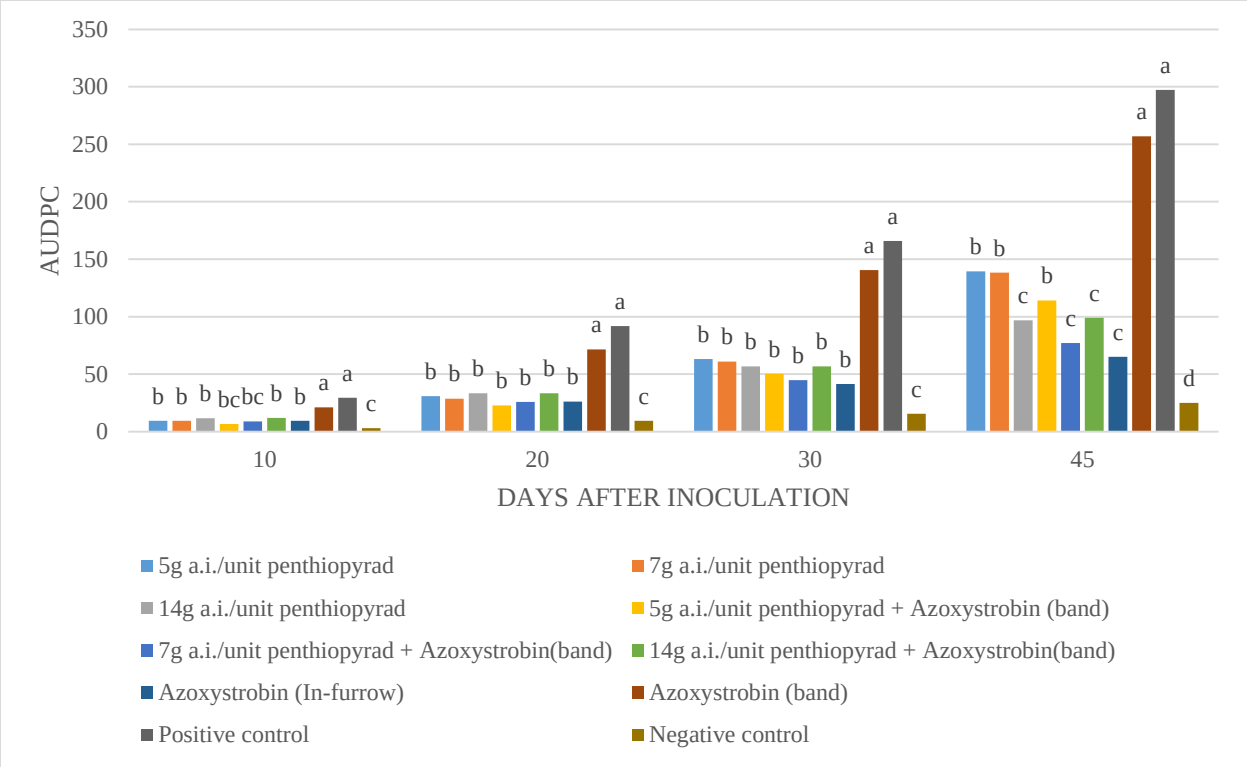


Figure 3.2: Effect of penthiopyrad seed treatments and/or azoxystrobin on disease development of *R. solani* AG 2-2 IIIB from 10 DAI to 45 DAI. 5, 7, and 14 g a.i. unit^{-1}: penthiopyrad was used as seed treatment at 5, 7, and 14 g a.i. unit^{-1}; azoxystrobin (in-furrow): azoxystrobin at 167 g a.i. ha^{-1} was applied in-furrow at planting; azoxystrobin (foliar): azoxystrobin at 167 g a.i. ha^{-1} was applied foliarly on 14 DAI. Treatments with the same number are not significantly different at $P \leq 0.05$ according to the contrast analysis of AUDPC between each two groups of treatments.

Discussion

R. solani is an ubiquitous soil-borne fungus that occurs more frequently and severely in sugarbeet-growing areas of Minnesota and North Dakota, where the most aggressive isolates *R. solani* AG 2-2 IIIB and IV are widely distributed (Brantner and Windels, 2007). This fungus initiates infection when the average daily soil temperature reaches the threshold of 18°C (Khan et al., 2010). Over the past six years, average daily soil temperature in sugarbeet fields in North Dakota and Minnesota reached 18°C when planting was done in May (NDAWN 2010, 2011, 2012

http://ndawn.ndsu.nodak.edu/). Therefore, early disease control is necessary for effectively managing *R. solani* in the sugarbeet field.

Our study showed that the use of resistant cultivar failed to provide effective disease control early in the season. Gaskill (1968) postulated that sugarbeet resistance is expressed against *R. solani* until four weeks after planting. Recent research showed that sugarbeet seeds to seedlings at three weeks after planting under controlled conditions in the greenhouse was the most vulnerable growth stage for *R. solani* infection, irrespective of the level of susceptibility of the cultivar to *R. solani* (See Chapter 4). In this study, *R. solani*-infested barley grain was placed close to each sugarbeet seed at planting, when Rhizoctonia resistance was not expressed in sugarbeet germplasm.

Early disease control of *R. solani* is achieved by applying azoxystrobin as in-furrow on sugarbeet seeds (Brantner and Windels, 2010; Stump et al., 2004; Windels and Brantner, 2005). In our study, azoxystrobin in-furrow provided effective control of *R. solani*, but the plant stand was significantly lower than the negative control. It is probably due to azoxystrobin negatively impacting germination and emergence of sugarbeet seeds. However, azoxystrobin applied foliarly failed to protect sugarbeet seedling that was inoculated with *R. solani* at 14 DAI. This is because azoxystrobin is ineffective at controlling *R. solani* after infection occurs (Brantner and Windels, 2001; Stump et al., 2004).

Penthiopyrad is a novel succinate dehydrogenase inhibitor (SDHI) fungicide that is effective against fungal diseases caused by basidiomycetes and ascomycetes (Yanase et al., 2007). In this study, penthiopyrad used as a seed treatment provided effective control of *R. solani* compared with the positive control and resulted in similar plant stand as the treatment where azoxystrobin was applied in-furrow during the first 30 days after inoculation. On 45 DAI, 14 g a.i.

unit^{-1} was still able to prevent *R. solani* infection whereas 5 and 7 g a.i. unit^{-1} were not as effective as azoxystrobin applied in-furrow since its efficacy was gradually lost and the plant survivors started to die on 30 DAI. Windels and Brantner (2009) showed that penthiopyrad seed treatment between 7 g and 84 g a.i. unit^{-1} were as effective on 28 DAI as azoxystrobin in managing *R. solani* AG 4, AG 2-2 IV, and AG 2-2 IIIB.

In this study, azoxystrobin was applied on 14 DAI after penthiopyrad seed treatment to improve the disease control over time. Azoxystrobin is known to be ineffective when infection has already occurred. The effectiveness of supplementing penthiopyrad seed treatment with azoxystrobin 14 DAI depends on whether the penthiopyrad treatment provided effective control until the azoxystrobin was applied. Azoxystrobin was ineffective following penthiopyrad seed treatment at 5 g a.i. unit^{-1}, probably because the rate was too low to prevent infection. The banded azoxystrobin significantly reduced AUDPC for 7 g a.i. unit^{-1} penthiopyrad on 45 DAI, resulting in similar disease control as 14 g a.i. unit^{-1} penthiopyrad. This would suggest that the 7 g a.i. unit^{-1} penthiopyrad rate was effective at preventing early infection which was continued with the azoxystrobin to day 45 DAI.

In North Dakota and Minnesota, some sugarbeet growers have adopted the practice of applying a starter fertilizer at planting. There is also a need to protect sugarbeet seedlings from damping-off which can be done by applying an in-furrow fungicide. However, the azoxystrobin (and pyraclostrobin) widely used in-furrow label does not recommend mixing with starter fertilizers for use together at planting because of the possibility of phytotoxicity. To avoid the use of a fertilizer and fungicide mixture, the fungicide can be used as a seed treatment, which allows growers to apply the fertilizer alone. Seed treatment is also cost-efficient and friendly to the ecosystem due to its lower dosage rate than in-furrow application.

Penthiopyrad has a different mode of action from azoxystrobin to prevent infection at high enough rates or suppress germination and growth of *R. solani,* and no cross-resistance has been observed between the SDHI and QoI class of fungicides (Avenot and Michailides, 2010). Consequently, penthiopyrad can be used as a seed treatment to effectively control *R. solani* early in the season and can be rotated with currently-used azoxystrobin to manage fungicide resistant issues.

Literature cited

Avenot, H.F., and Michailides, T.J. 2010. Progress in understanding molecular mechanisms and evolution of resistance to succinate dehydrogenase inhibiting (SDHI) fungicides in phytopathogenic fungi. Crop Prot. 29:643–651.

Brantner, J.R., and Windels. C.E. 2001. Band and broadcast applications of quadris for control of Rhizoctonia root and crown rot on sugarbeet. SBREB. 32:282-286. Available at http://www.sbreb.org/research/plant/plant01/Band_and_Broadscast.doc. (Verified 11 Mar. 2015).

Brantner, J.R., and Windels, C.E. 2007. Distribution of *Rhizoctonia solani* AG 2-2 intraspecific groups in the Red River Valley and southern Minnesota. SBREB. 38:242–246. Available at http://www.sbreb.org/research/plant/plant07/08DistributionOfRhizocRRV.pdf. (Verified 11 Mar. 2015).

Brantner, J.R., and Windels, C.E. 2010. Efficacy of in-furrow and postemergence fungicides in controlling Rhizoctonia on sugarbeet. SBREB. 41:246-249. http://www.sbreb.org/research /plant/plant10/BratnerEfficacyOfInFurrow2010.pdf. (Verified 11 Mar. 2015).

FAO. 2014. FAO Statistical Yearbook 2014. Available at http://www.fao.org/3/a-i3621e.pdf. (Accessed 11 Mar. 2015).

Gaskill, J.O. 1968. Breeding for Rhizoctonia resistance in sugarbeet. Am. Soc. Sugarbeet Technol. 15:107-119.

Khan, M.F.R., and Carlson, A.L. 2009. Effect of fungicides on controlling Rhizoctonia crown and root rot in sugarbeet. SBREB. 40:246-247. Available at http://www.sbreb.org/research /plant/plant09/EffecFfungicidesControllingRhizoctonia2009t.pdf. (Verified 11 Mar. 2015)

Khan, M.F.R., Khan, J., and Bradley, C.A. 2010. Effect of azoxystrobin applications based on soil temperature on Rhizoctonia root and crown rot of sugarbeet. Int. Sugar J. 112:557-560.

Kirk, W.W., Wharton, P.S., Schafer, R.L., Tumbalam, P., Poindexter, S., Guza, C., Fogg, R., Schlatter, T., Stewart, J., Hubbell, L., and Ruppal, D. 2008. Optimizing fungicide timing

for the control of Rhizoctonia crown and root rot of sugarbeet using soil temperature and plant growth stages. Plant Dis. 92:1091-1098.

Niehaus, W.S. 2013. Results of American Crystal's 2013 official coded variety trails. SBREB. 42:276-346. Available at http://www.sbreb.org/research/sugar/sugar13/ResultsAmerican Crystal.pdf. (Verified 11 Mar. 2015).

Noor, A., and Khan, M.F.R. 2014. Efficacy and safety of mixing azoxystrobin and starter fertilizers for controlling *Rhizoctonia solani* in sugarbeet. Phytoparasitica 43:51-55.

Olaya, G., Buitrago, C., Pearsaul, D., Sierotzki, H., and Tally, A. 2012. Detection of resistance to QoI fungicides in *Rhizoctonia solani* isolates from rice. Phytopathology 102: S4.88.

Shaner, G., and Finney, R.E. 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. Phytopathology 67:1051-1056.

Stachler, J.M., Carlson, A.L., Luecke, J.L., Boetel, M.A., and Khan, M.F.R. 2009. Survey of weed control and production practices on sugarbeet in Minnesota and eastern North Dakota in 2009. SBREB. 40:39-60. Available at http://www.sbreb.org/research/weed/weed09 /SurveyWeedControl2009.pdf. (Verified 11 Mar. 2015).

Stump, W.L., Franc, G.D., Harveson, R.M., and Wilson, R.G. 2004. Strobilurin fungicide timing for Rhizoctonia root and crown rot suppression in sugarbeet. JSBR. 41:17-37.

USDA-ERS. 2014. Sugar and sweeteners yearbook tables. Available at http://www.ers.usda.gov /datafiles/Sugar_and_Sweeteners_Yearbook_Tables/US_Sugar_Supply_and_Use/TABL E14.XLS. (Verified 11 Mar. 2015).

Windels, C.E., and Brantner, J.R. 2005. Early-season application of azoxystrobin to sugarbeet for control of *Rhizoctonia solani* AG-4 and AG 2-2. JSBR. 42:1-16.

Windels, C.E., and Brantner, J.R. 2009. Penthiopyrad seed treatment on sugarbeet for control of *Rhizoctonia solani*. SBRER. 40:237-239. Available at http://www.sbreb.org/research/plant /plant09/PenthiopyradSeedTreatment2009.pdf. (Verified 11 Mar. 2015).

Yanase, Y., Yoshikawa, Y., Kishi, J., and Katsuta, H. 2007. The history of complex II inhibitors and the discovery of penthiopyrad. In H. Ohkawa, H. Miyagawa, and P.W. Lee (Eds.), Pesticide Chemistry: Crop Protection, Public Health, Environmental Safety (pp. 295-303). Wiley, New Jersey, NY, USA.

CHAPTER 4. AGE OF SUGARBEET SEEDLINGS AND RESISTANCE TO *RHIZOCTONIA SOLANI*

Introduction

Rhizoctonia solani is a soil-borne plant pathogen widely distributed around the world, causing root diseases on many important economic crops (Anderson, 1982). This fungus consists of genetically different isolated population called anastomosis groups (AGs), which can be further subdivided into subgroups with different host ranges (Ogoshi, 1987). *R. solani* AG 2-2 is mainly responsible for Rhizoctonia damping-off, crown and root rot on sugarbeet (*Beta vulgaris* L.). *R. solani* AG 2-2 IIIB and IV are the most virulent subgroups and AG 2-2 IIIB seems to be more aggressive than AG 2-2 IV (Bolton et al., 2010). Damping-off occurs when infected seedlings have brown to black lesions formed on the hypocotyl and eventually collapse. Crown rot infection typically starts on the sugarbeet crown, but can also occur at or below the soil line. Root rot develops as brown to black, sunken, circular lesions, and these lesions often coalesce to cover large area of root surface. Above-ground symptoms include severe wilting and chlorosis of leaves with black lesions on the base of the petioles (Harveson et al., 2009).

Rhizoctonia solani is reported to affect 24% of sugarbeet growing area in the United States and has increased disease pressure in Europe (Buhre et al., 2009; Windels, et al., 2009). The yield loss caused by this disease varies greatly from field to field, but significant loss can occur when weather conditions are favorable. Research has shown that entire sugarbeet plots can be lost to the disease (Khan et al., 2010; Windels and Branter, 2005) and sometimes entire fields have to be destroyed because of the high severity of the disease across the entire field (Khan, personal communication). Rhizoctonia was listed as the most serious production problem by sugarbeet growers in Minnesota and North Dakota according to an annual grower's survey (Stachler et al.,

2009). Cultural practices such as crop rotation and cultivation provide limited protection to sugarbeet plants. QoI fungicides are widely used by sugarbeet growers to provide effective control of *R. solani* (Carlson et al., 2012), but fungicide resistance issue is a concern since the reported resistance of *R. solani* to azoxystrobin on rice in Louisiana (Olaya et al., 2012).

Developing resistance in sugarbeet to *R. solani* started in the late 1950s, and a number of resistant germplasms were released in the next forty years (Hecker and Gaskill, 1972; Hecker and Smith, 1979; Hecker and Ruppel, 1983 and 1986; Panella, 1995). Resistance to *R. solani* was described as a quantitative trait, which is conditioned by two major loci and several minor modifying genes (Hecker and Ruppel, 1975; Panella, 2005). There is no commercial resistant variety which is immune to *R. solani*. Also, the resistance against *R. solani* damping-off is difficult to be screened due to the high mortality of seedlings in artificial inoculation (Gaskill, 1968). Engelkes and Windels (1994) reported that disease severity caused by *R. solani* AG 2-2 decreased as plant age increased. It is not known at what age sugarbeet plants initiates or express resistance to *R. solani*. It will be useful to know the plant stage at which sugarbeet becomes resistant, so that growers can target the susceptible stage with adequate fungicide protection. Therefore, the objective of this research was to determine at what growth stage sugarbeet plants express resistance to *R. solani*.

Materials and methods

Experiments were conducted in a greenhouse, at North Dakota State University, Fargo, ND, USA. Plastic trays measuring 27 x 13 x 13 (T. O. Plastics Inc.; Clearwater, MN, USA) were filled with Sunshine mix 1 peat (Sun Gro Horticulture Ltd.; Alberta, Canada) and 20 g fertilizer Osmocote 15-9-12 (Scotts Company; Marysville, OH). Isolates of *R. solani* AG 2-2 IIIB recovered from sugarbeet were obtained from Dr. Carol Windels, University of Minnesota. Pure cultures of

R. solani AG 2-2 IIIB were used to produce inoculum by inoculating sterilized barley as described by Noor and Khan (2014). Seed companies provided cultivars with varying levels of susceptibility to *R. solani*. Syngenta, Hilleshog (Moorhead, MN, USA) provided Hilleshog 4022RR (susceptible cultivar), Hilleshog 4195RR (moderately resistant cultivar), and Hilleshog 4012RR (resistant cultivar); Betaseed, Inc. (Shakopee, MN, USA) provided BTS 89RR50 (susceptible cultivar), BTS 80RR52 (moderately resistant cultivar), and BTS 89RR83 (resistant cultivar); SesVanderHave (SES) (Fargo, ND, USA) provided three sugarbeet cultivars (proprietary materials) that were susceptible, moderately resistant, and resistant to *R. solani*.

Sugarbeet seeds were placed in 2.5-cm-deep furrows in the middle of each tray. Each cultivar was planted at one week interval to produce plants from seed up to 10 weeks old, which were simultaneously inoculated with *R. solani*-infested barley grain. Inoculation was conducted by placing one inoculated barley grain 1-cm to the side of each of ten sugarbeet seeds in the furrow or to each plant (Noor and Khan, 2014). The negative control was also included for each cultivar using sterilized barley but without *R. solani*. Greenhouse conditions were set to allow light for 12-hour photoperiod with a temperature range of 20 ± 2°C. Watering was done as required to maintain adequate soil moisture for plant growth and disease development. Twenty eight days after inoculation, sugarbeet roots at different growth stages were carefully hand-harvested and washed under tap water. Disease severity was evaluated using a 0 to 7 scale: 0 (no disease), 1 (crown area slightly scurfy), 2 (<5% infection), 3 (6-25% infection), 4 (26-50% infection), 5 (51-75% infection), 6 (>75% infection), and 7 (the root completely deteriorated or dead plant) (Windels and Nabben-Schindler, 1996).

The experimental layout was a split-plot design, with cultivar as the whole-plot factor and inoculation timing as the sub-plot factor. The trial was repeated once with three replicates. Disease

severity data were obtained by calculating an averaged disease scale from ten individual plants in each tray. Data were analyzed by non-parametric method using the SAS procedures of Proc Rank and Proc Mixed (Version 9.3, SAS Institute Inc.; Cary, NC, USA). The relative effects of disease severity for each treatment and their confidence intervals at 95% confidence were calculated using 'F1_LD_F1' and 'LD_CI' macros (Shah and Madden, 2004).

Results

No significant differences in disease severity were observed between sugarbeet cultivars, while plant age had a significant effect on disease severity (Table 4.1). The negative controls for each cultivar were without root symptoms of Rhizoctonia damping-off, crown and root rot and therefore had the lowest disease severity (Table 4.2). Sugarbeet cultivars inoculated at the seed stage, and 1, 2, and 3 weeks after planting were highly susceptible to *R. solani* and their disease severities were not significantly different from each other. Even in resistant cultivars, the plant stand was low since damping-off killed almost all seedlings. Compared with the period between the seed stage and 3 weeks after planting, there was a trend for resistant and moderately resistant cultivars inoculated at 4 and 5 weeks after planting became more resistant to *R. solani* with lower disease severity. Six cultivars, 4194RR, 4012RR, 89RR83, 4022RR, SES susceptible cultivar, and SES resistant cultivar, had significantly lower disease severity, and 4012RR and 4195RR had similar disease severity as the negative controls. For sugarbeet cultivars inoculated at 6 weeks or more weeks after planting, disease severities from all the cultivars were not significantly different from each other, but they were significantly different from the negative controls (Table 4.2; Figure 4.1).

In Figure 4.1, three cultivars with different levels of *R. solani* susceptibility from the same company were compared. The moderately resistant and resistant cultivars (4195RR and 4012RR)

from Syngenta, Hilleshog had similar resistance, while the susceptible cultivar (4022RR) had higher disease severity which decreased over time (Figure 4.1.A). Three cultivars from Betaseed, Inc. had similar levels of resistance, except for resistant cultivar (89RR83) from 4 to 7 weeks old which had significantly lower disease severity than the other two cultivars (89RR50 and 89RR52) (Figure 4.1.B). For SES, resistant cultivar had higher resistance than the other cultivars, but this difference was not significantly different when plants were inoculated at 10 weeks old (Figure 4.1.C).

Table 4.1: Test statistics for the effects of plant age and resistant level on the disease severity of sugarbeet roots by *R. solani* AG 2-2 IIIB.

Effect	ANOVA-type statistic (ATS)			
	df_N[a]	df_D[b]	ATS	*P* value
Cultivar	7.74	43.33	0.94	0.478
Plant age	4.65	∞	227.04	<0.001**
Cultivar × Plant age	20.51	∞	3.27	<0.001**

[a]df_N=numerator degrees of freedom.
[b]df_D=denominator degrees of freedom.
** Significantly different at $P \leq 0.001$.

Table 4.2: Effect of plant ages and cultivars on disease severity of sugarbeet caused by *R. solani* AG 2-2 IIIB.

Cultivar	Resistance level[a]	Plant age[b]	MDS[c]	REDS[d]	95% CI[e]
4022RR	S	seed	7.0	0.803	0.721-0.865
4022RR	S	1 week old	7.0	0.754	0.606-0.859
4022RR	S	2 week old	7.0	0.704	0.491-0.853
4022RR	S	3 week old	7.0	0.778	0.663-0.861
4022RR	S	4 week old	5.5	0.578	0.369-0.762
4022RR	S	5 week old	4.4	0.439	0.278-0.615
4022RR	S	6 week old	5.8	0.562	0.441-0.676
4022RR	S	7 week old	5.5	0.532	0.407-0.653
4022RR	S	8 week old	4.4	0.442	0.277-0.621
4022RR	S	9 week old	4.8	0.478	0.334-0.626
4022RR	S	10 week old	3.8	0.431	0.309-0.561
4022RR	S	No Inoculation	0.0	0.072	0.058-0.089
4195RR	MR	seed	7.0	0.709	0.494-0.857
4195RR	MR	1 week old	7.0	0.780	0.662-0.865
4195RR	MR	2 week old	7.0	0.722	0.509-0.865
4195RR	MR	3 week old	7.0	0.758	0.608-0.862
4195RR	MR	4 week old	0.1	0.184	0.081-0.369
4195RR	MR	5 week old	0.7	0.220	0.130-0.349
4195RR	MR	6 week old	1.9	0.266	0.180-0.376
4195RR	MR	7 week old	2.2	0.291	0.182-0.431
4195RR	MR	8 week old	2.5	0.314	0.200-0.456
4195RR	MR	9 week old	2.7	0.324	0.214-0.459
4195RR	MR	10 week old	3.1	0.364	0.267-0.474
4195RR	MR	No Inoculation	0.0	0.072	0.058-0.089
4012RR	R	seed	7.0	0.750	0.586-0.863
4012RR	R	1 week old	7.0	0.763	0.613-0.867
4012RR	R	2 week old	7.0	0.784	0.593-0.899
4012RR	R	3 week old	7.0	0.776	0.652-0.865
4012RR	R	4 week old	0.0	0.165	0.082-0.309
4012RR	R	5 week old	0.8	0.180	0.105-0.294
4012RR	R	6 week old	1.0	0.234	0.154-0.341
4012RR	R	7 week old	2.7	0.298	0.212-0.402
4012RR	R	8 week old	1.7	0.275	0.156-0.437
4012RR	R	9 week old	2.5	0.333	0.223-0.466
4012RR	R	10 week old	2.3	0.310	0.221-0.416
4012RR	R	No Inoculation	0.0	0.072	0.058-0.089
89RR50	S	seed	7.0	0.827	0.745-0.886

Table 4.2: Effect of plant ages and cultivars on disease severity of sugarbeet caused by *R. solani* AG 2-2 IIIB (continued).

Cultivar	Resistance level[a]	Plant age[b]	MDS[c]	REDS[d]	95% CI[e]
89RR50	S	1 week old	7.0	0.805	0.668-0.893
89RR50	S	2 week old	7.0	0.863	0.838-0.885
89RR50	S	3 week old	7.0	0.756	0.608-0.860
89RR50	S	4 week old	3.3	0.446	0.215-0.703
89RR50	S	5 week old	5.9	0.589	0.443-0.720
89RR50	S	6 week old	4.1	0.414	0.259-0.590
89RR50	S	7 week old	5.3	0.508	0.313-0.700
89RR50	S	8 week old	4.5	0.470	0.329-0.616
89RR50	S	9 week old	4.2	0.455	0.304-0.616
89RR50	S	10 week old	3.7	0.400	0.271-0.544
89RR50	S	No Inoculation	0.0	0.072	0.058-0.089
80RR52	MR	seed	7.0	0.772	0.649-0.860
80RR52	MR	1 week old	7.0	0.827	0.747-0.886
80RR52	MR	2 week old	7.0	0.746	0.587-0.858
80RR52	MR	3 week old	7.0	0.740	0.531-0.876
80RR52	MR	4 week old	7.0	0.663	0.449-0.826
80RR52	MR	5 week old	5.3	0.531	0.352-0.701
80RR52	MR	6 week old	3.4	0.395	0.261-0.547
80RR52	MR	7 week old	4.0	0.410	0.284-0.549
80RR52	MR	8 week old	3.2	0.389	0.263-0.533
80RR52	MR	9 week old	4.6	0.494	0.322-0.667
80RR52	MR	10 week old	2.9	0.367	0.244-0.512
80RR52	MR	No Inoculation	0.0	0.072	0.058-0.089
89RR83	R	seed	7.0	0.816	0.707-0.890
89RR83	R	1 week old	7.0	0.827	0.744-0.887
89RR83	R	2 week old	7.0	0.827	0.744-0.887
89RR83	R	3 week old	7.0	0.780	0.654-0.869
89RR83	R	4 week old	1.9	0.329	0.165-0.552
89RR83	R	5 week old	2.0	0.302	0.164-0.490
89RR83	R	6 week old	1.7	0.279	0.178-0.412
89RR83	R	7 week old	1.8	0.275	0.183-0.392
89RR83	R	8 week old	4.2	0.504	0.313-0.694
89RR83	R	9 week old	2.7	0.352	0.204-0.536
89RR83	R	10 week old	2.3	0.400	0.276-0.538
89RR83	R	No Inoculation	0.0	0.072	0.058-0.089
SES[f]	S	seed	7.0	0.746	0.590-0.857
SES	S	1 week old	7.0	0.827	0.746-0.886
SES	S	2 week old	7.0	0.735	0.562-0.857

Table 4.2: Effect of plant ages and cultivars on disease severity of sugarbeet caused by *R. solani* AG 2-2 IIIB (continued).

Cultivar	Resistance level[a]	Plant age[b]	MDS[c]	REDS[d]	95% CI[e]
SES	S	3 week old	7.0	0.731	0.546-0.859
SES	S	4 week old	4.9	0.506	0.264-0.745
SES	S	5 week old	3.8	0.423	0.282-0.578
SES	S	6 week old	4.8	0.499	0.378-0.621
SES	S	7 week old	6.2	0.631	0.515-0.734
SES	S	8 week old	4.6	0.502	0.382-0.621
SES	S	9 week old	4.4	0.459	0.362-0.560
SES	S	10 week old	5.5	0.525	0.404-0.643
SES	S	No Inoculation	0.0	0.072	0.058-0.089
SES	MR	seed	7.0	0.747	0.583-0.861
SES	MR	1 week old	7.0	0.827	0.745-0.887
SES	MR	2 week old	7.0	0.735	0.559-0.858
SES	MR	3 week old	7.0	0.733	0.546-0.861
SES	MR	4 week old	1.5	0.345	0.176-0.566
SES	MR	5 week old	2.6	0.394	0.222-0.597
SES	MR	6 week old	4.3	0.461	0.307-0.623
SES	MR	7 week old	6.5	0.633	0.505-0.745
SES	MR	8 week old	4.9	0.498	0.367-0.630
SES	MR	9 week old	4.7	0.475	0.376-0.575
SES	MR	10 week old	4.0	0.435	0.322-0.556
SES	MR	No Inoculation	0.0	0.072	0.058-0.089
SES	R	seed	7.0	0.827	0.745-0.886
SES	R	1 week old	7.0	0.827	0.745-0.886
SES	R	2 week old	7.0	0.827	0.745-0.886
SES	R	3 week old	7.0	0.746	0.581-0.861
SES	R	4 week old	5.6	0.542	0.312-0.756
SES	R	5 week old	0.5	0.214	0.127-0.341
SES	R	6 week old	2.1	0.300	0.187-0.445
SES	R	7 week old	4.5	0.470	0.350-0.593
SES	R	8 week old	1.1	0.275	0.145-0.461
SES	R	9 week old	2.7	0.334	0.212-0.485
SES	R	10 week old	1.8	0.262	0.153-0.413
SES	R	No Inoculation	0.0	0.072	0.058-0.089

[a]S, MR, and R indicated that sugarbeet cultivars were susceptible, moderately resistant, or resistant to *R. solani*, respectively.

[b]Inoculation was done at different plant ages from seed to 10 weeks old; 'No Inoculation' represents the negative control where sterilized barley was placed.

[c]Median disease rank.

[d]Relative effects of disease severity.
[e]Upper-lower values of 95% confidence interval (CI) for treatment relative effect.
[f]SES represents SesVanderHave.

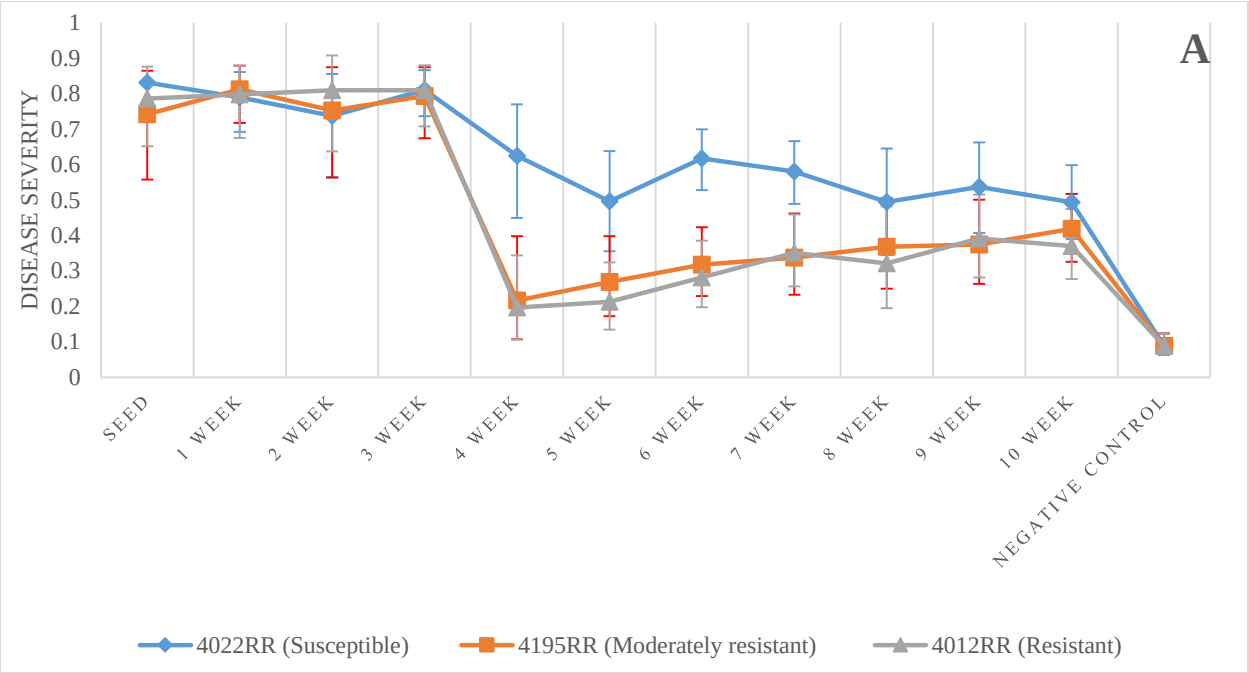
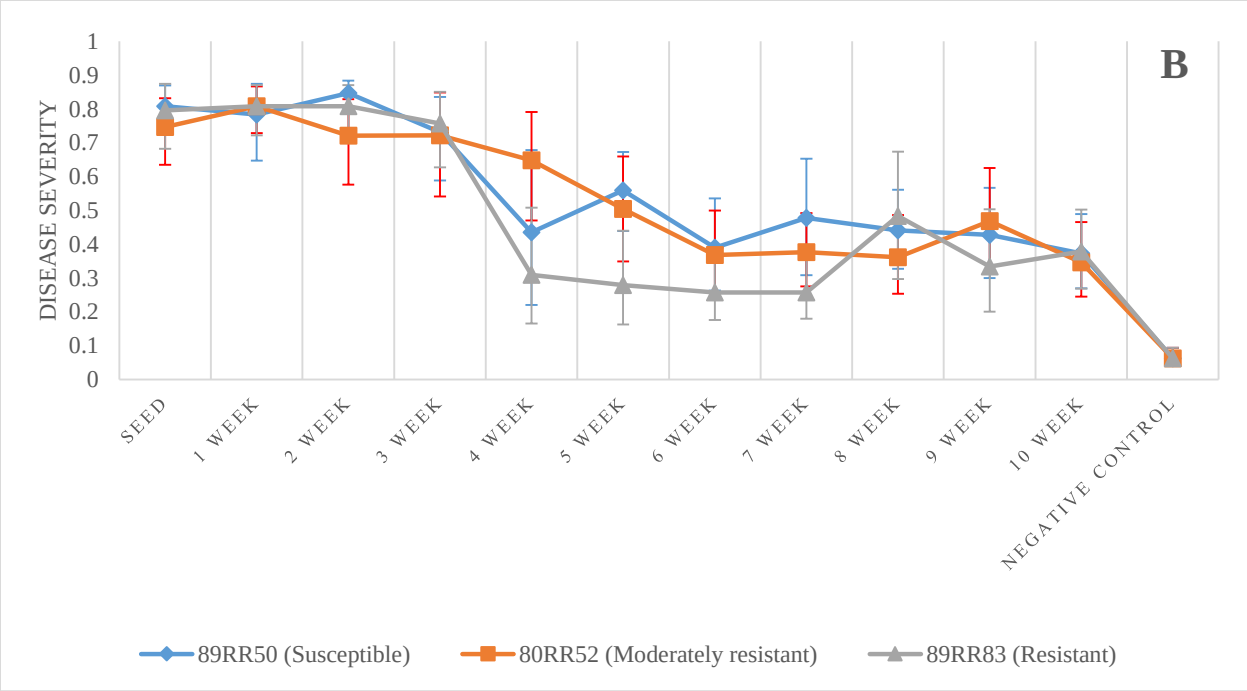


Figure 4.1: Effects of resistant level and plant age on disease severity caused by *R. solani* AG 2-2 IIIB on sugarbeet. Inoculation with *R. solani* was simultaneously conducted on sugarbeet from seed to ten weeks old, while the negative controls were also inoculated using sterilized barley without *R. solani*. A) 4012RR, 4195RR, and 4012RR from Syngenta, Hilleshog; B) 89RR50, 80RR52, and 89RR53 from Betaseed Inc. C) Three cultivars with three different resistant levels to *R. solani* from SES (SesVanderHave).



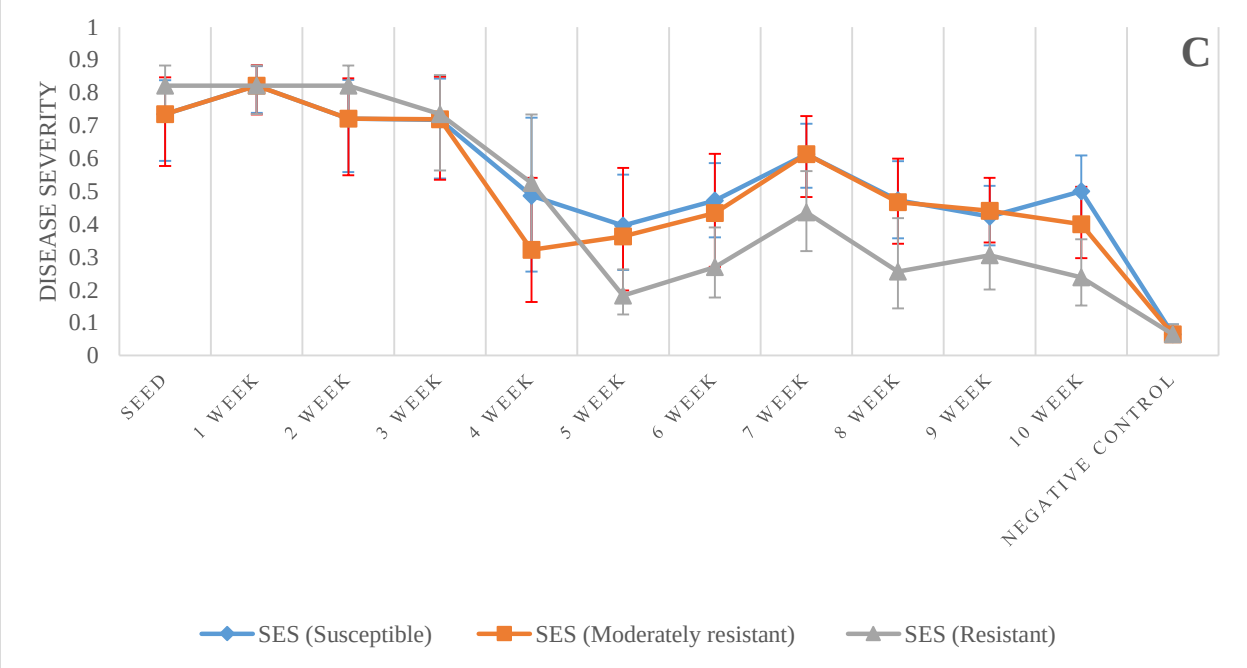


Figure 4.1: Effects of resistant level and plant age on disease severity caused by *R. solani* AG 2-2 IIIB on sugarbeet (continued). Inoculation with *R. solani* was simultaneously conducted on sugarbeet from seed to ten weeks old, while the negative controls were also inoculated using sterilized barley without *R. solani*. A) 4012RR, 4195RR, and 4012RR from Syngenta, Hilleshog; B) 89RR50, 80RR52, and 89RR53 from Betaseed Inc. C) Three cultivars with three different resistant levels to *R. solani* from SES (SesVanderHave).

Discussion

Nine sugarbeet cultivars from three commercial companies were evaluated to determine at what age after planting they expressed resistance to *R. solani* by preventing severe infection from taking place. None of the cultivars expressed a good level of resistance until they were 4 weeks in age after planting. In the greenhouse, it should be noted that emergence takes place in about 5 days after planting with cotyledonary beets after one week. Each week, thereafter, about two new leaves are produced. Inoculation of seeds and seedlings up to 3 weeks in age resulted in severe damping-off which killed most of the seedlings after emergence compared with the negative controls. This result was consistent with Gaskill's postulate (1968) who reported that *R. solani* was too severe when inoculation was conducted on young sugarbeet plants to use them to evaluate varietal resistance. Minnesota and North Dakota contribute about 58% of the nation's sugarbeet tonnage. In this area, sugarbeet seeds were planted when the temperature was above 18°C at 10-cm soil depth for the past several years (NDAWN 2010, 2011, 2012; http://ndawn.ndsu.nodak.edu/). *Rhizoctonia solani* becomes infective in warm conditions and in the presence of adequate moisture (Khan et al., 2010). Therefore, the practice of using fungicide in-furrow application or seed treatment could be a reliable way to protect sugarbeet plants when they are at the most vulnerable stage to *R. solani* early in the season.

Based on this study, sugarbeet plants started to express resistance to *R. solani* when plants were at least four weeks old (6-8 leaf stage). All sugarbeet cultivars had lower disease severity at the 6- to 8-leaf stage and the cultivars labeled as resistant had lower disease severity than the cultivars labeled as susceptible. The sugarbeet plants under the greenhouse growing conditions were at the 6- to 8-leaf growth stage, which could be a preliminary indicator as when resistant cultivars no longer need to be protected with fungicides.

Sugarbeet at 6- to 8-leaf stage was considered as mature plants (Karaoglanidis and Karadimos, 2006; Whitney and Duffus, 1986). The resistance level was significantly different between seedlings and mature plants for the same *R. solani* AG (Bolton et al., 2010). Similarly, soybean seedlings are highly susceptible to *R. solani* and become resistant at 2 or 3 weeks after planting. Increased resistance of older soybean plants resulted from a conversion by adding calcium to pectin in the cell component which renders pectin material more resistant to polygalacturonase (Bateman and Lumsden, 1965). A number of enzymes, such as pectolytic enzymes and polygalacturonase are involved in the infection process and pathogenicity of *R. solani* (Bateman, 1963; Brookhouser et al., 1980; Sherwood, 1966). In sugarbeet plants, pectin lyase produced by *R. solani* AG 2-2 strain might play a role in decomposition of the cell wall barrier and invasion of the fungus since the injection of purified pectin lyase caused wilt on susceptible sugarbeet plants but not on resistant ones (Bugbee, 1990). A protein inhibitor of pectin lyase was present at higher concentration in rotted and adjacent tissue than healthy tissue, as well as resistant cultivars than susceptible cultivars (Bugbee, 1993).

No commercial of sugarbeet cultivar is immune to *R. solani*. This study showed that *R. solani* inoculation on adult sugarbeet plants resulted in 5 to 24% of decay area on the root surface of resistant cultivars. This disease severity might not be biologically important for the survival of sugarbeet (Engelkes and Windels, 1994). As the plants became older, they became more resistant to *R. solani*. Some seed companies, such as Syngenta, Hilleshog produced cultivars where moderately resistant and resistant cultivars provided distinct expression of resistance after 4 weeks of planting compared with the susceptible cultivars. Even the susceptible cultivars became less susceptible at 4 weeks after planting. This study demonstrated that sugarbeet cultivars were the most susceptible to *R. solani* until they reached the 6- to 8-leaf stage. As such, in areas with a

history of high *R. solani* disease severity, the practice of using a fungicides such as penthiopyrad or azoxystrobin in-furrow or penthiopyrad as a seed treatment as previously discussed (Chapters 2 and 3) could be used to prevent infection of sugarbeet at a vulnerable stage.

Literature cited

Anderson, N.A. 1982. The genetics and pathology of *Rhizoctonia solani*. Annu. Rev. Phytopathol. 20:329-347.

Bateman, D.F. 1963. Pectolytic activities of culture filtrates of *Rhizoctonia solani* and extracts of Rhizoctonia-infected tissues of beans. Phytopathology 53:197-204.

Bateman, D.F., and Lumsden, R.D. 1965. Relation of calcium content and nature of the pectic substances in bean hypocotyls of different ages to susceptibility to and isolate of *Rhizoctonia solani*. Phytopathology 55:734-38.

Bolton, M.D., Panella, L., Campbell, L., and Khan, M.F.R. 2010. Temperature, moisture, and fungicide effects in managing Rhizoctonia root and crown rot of sugarbeet. Phytopathology 100:689–697.

Brookhouser, L.W., Hancock, J.G., and Weinhold, D.A. 1980. Characterization of endopolygalacturonase produced by *Rhizoctonia solani* in culture and during infection of cotton seedlings. Phytopathology 70:1039-1042.

Bugbee, W.M. 1990. Purification and characteristics of pectin lyase from *Rhizoctonia solani*. Physiol. Mol. Plant Path. 36:15-25.

Bugbee, W.M. 1993. A pectin lyase inhibitor protein from cell walls of sugarbeet. Phytopathology 83:63-68.

Buhre, C., Kluth, C., Bürcky, K., Märländer, B., and Varrelmann, M. 2009. Integrated control of root and crown rot in sugarbeet: Combined effects of cultivar, crop rotation, and soil tillage. Plant Dis. 93:155-161.

Carlson, A.L., Khan, M.F.R., Stachler, J.M., and Boetel, M.A. 2012. Survey of fungicide use in sugarbeet in Minnesota and eastern North Dakota in 2012. SBREB. 42:181-182. Available at http://www.sbreb.org/research/plant/plant11/CarlsonSurveyFungicide2012.pdf. (Verified 11 Mar. 2015).

Engelkes, C.A., and Windels, C.E. 1994. Relationship of plant age, cultivar, and isolate of *Rhizoctonia solani* AG 2-2 to sugarbeet and crown rot. Plant Dis. 78:685-689.

Gaskill, J.O. 1968. Breeding for Rhizoctonia resistance in sugarbeet. J. Am. Soc. Sugarbeet Technol. 15:107-119.

Harveson, R.M., Hanson, L.E., and Hein, G.L. (Eds.). 2009. Compendium of beet diseases and pests. The American Phytopathological Society, St. Paul, MN, USA.

Hecker, R.J., and Ruppel, E.G. 1975. Inheritance of resistance to Rhizoctonia root rot sugarbeet. Crop Sci. 15:487-490.

Hecker, R.J. and Gaskill, J.O. 1972. Registration of FC701 and FC702 sugarbeet germplasm. Crop Sci. 12:400.

Hecker, R.J. and Smith, G.A. 1979. Registration of FC704 sugarbeet germplasm. Crop Sci. 19:934-935.

Hecker, R.J. and Ruppel, E.G. 1983. Registration of FC711 sugarbeet germplasm. Crop Sci. 23:601-602.

Hecker, R.J. and Ruppel, E.G. 1986. Registration of Rhizoctonia root rot resistant sugarbeet germplasm FC712. Crop Sci. 26:213-214.

Karaoglanidis, G.S., and Karadimos, D.A. 2006. Efficacy of strobilurins and mixtures with DMI fungicides in controlling powdery mildew in field-grown sugarbeet. Crop Prot. 25:977-983.

Khan, M.F.R., Khan, J., and Bradley, C.A. 2010. Effect of azoxystrobin applications based on soil temperature on Rhizoctonia root and crown rot of sugarbeet. Int. Sugar J. 112:557-560.

Noor, A., and Khan, M.F.R. 2014. Efficacy and safety of mixing azoxystrobin and starter fertilizers for controlling Rhizoctonia solani in sugarbeet. Phytoparasitica 43:51-55.

Ogoshi, A. 1987. Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani* Kühn. Annu. Rev. Phytopathol. 25:125-143.

Olaya, G., Buitrago, C., Pearsaul, D., Sierotzki, H., and Tally, A. 2012. Detection of resistance to QoI fungicides in *Rhizoctonia solani* isolates from rice. Phytopathology 102: S4.88.

Panella, L. 2005. Root rots. In: E. Biancardi, L. G. Campbell, G. N. Skaracis, and M. de Biaggi (Eds.), Genetics and Breeding of Sugarbeet (pp. 95-100). CRC Press, Boca Raton, FL, USA.

Panella, L., Ruppel, E.G., and Hecker, R.J. 1995. Registration of four multigerm sugarbeet germplasms resistant to Rhizoctonia root rot: FC 716, FC 717, FC 718, and FC 719. Crop Sci. 35:291-292.

Shah, D.A., and Madden, L.V. 2004. Nonparametric analysis of ordinal data in designed factorial experiments. Phytopathology 94:33-43.

Sherwood, R.T. 1966. Pectin lyase and polygalacturonase production by *Rhizoctonia solani* and other fungi. Phytopathology 56:279-286.

Stachler, J.M., Carlson, A.L., Luecke, J.L., Boetel, M.A., and Khan, M.F.R. 2009. Survey of weed control and production practices on sugarbeet in Minnesota and eastern North Dakota. SBREB. 40: 39-60. Available at http://www.sbreb.org/research/weed/weed09/SurveyWeedControl2009.pdf. (Verified 11 Mar. 2015).

Whitney, E.D., and Duffus, J.E. 1986. Compendium of beet diseases and insects. The American Phytopathological Society, St. Paul, MN, USA.

Windels, C.E., and Brantner, J.R. 2005. Early-season application of azoxystrobin to sugarbeet for control of *Rhizoctonia solani* AG 4 and AG 2-2. JSBR. 42:1-17.

Windels, C.E., Jacobsen, B.J., and Harveson, R.M. 2009. Rhizoctonia root and crown rot. In R.M. harveson, L.E. Hanson, and G.L. Hein (Eds.), Compendium of Beet Diseases and Pests (pp. 33-36). The American Phytopathological Society Press, St. Paul, MN, USA.

Windels, C.E., and Nabben-Schindler, D.J. 1996. Limitations of a greenhouse bioassay for determining potential of Aphanomyces root rot in sugarbeet fields. JSBR. 33:1-13.